YU ISSN 0042-8450

ВОЈНОСАНИТЕТСКИ ПРЕГЛЕД

Часопис лекара и фармацеута Војске Србије

Military Medical and Pharmaceutical Journal of Serbia

Vojnosanitetski pregled

Vojnosanit Pregl 2026; July Vol. 83 (No. 7): pp. 409–470.

2026 July Vol. 83 (No. 7): pp. 409–470.
Vojnosanitetski Pregled

Vol. 83, No.7, 2026

VOJNOSANITETSKI PREGLED

The first issue of *Vojnosanitetski pregled* was published in September 1944
The Journal continues the tradition of Vojno-sanitetski glasnik which was published between 1930 and 1941

PUBLISHER
Ministry of Defence of the Republic of Serbia, University of Defence, Belgrade, Serbia

ISSN 0042-8450
eISSN 2406-0720
Open Access
(CC BY-SA)

Editorial Office: University of Defence, Faculty of Medicine of the Military Medical Academy, Center for Medical Scientific Information, Crnotravska 17, 11 040 Belgrade, Serbia. E-mail: **vsp@vma.mod.gov.rs**

Papers published in the Vojnosanitetski pregled are indexed in: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, SCOPUS, Excerpta Medica (EMBASE), Google Scholar, EBSCO, Biomedicina Serbica, Serbian Citation Index (SCIndex), DOAJ. Contents are published in Giornale di Medicine Militare and Revista de Medicina Militara. Reviews of original papers and abstracts of contents are published in International Review of the Armed Forces Medical Services.

The Journal is published monthly. Subscription: Giro Account No. 840-19540845-28, refer to number 122742313338117. To subscribe from abroad phone to +381 11 3608 997. Subscription prices per year: individuals 5,000.00 RSD, institutions 10,000.00 RSD, and foreign subscribers 150 €.

Printed by: Vojna štamparija, Beograd, Resavska 40b

Vol. 83, br. 7, 2026

VOJNOSANITETSKI PREGLED

Prvi broj *Vojnosanitetskog pregleda* izašao je septembra meseca 1944. godine
Časopis nastavlja tradiciju *Vojno-sanitetskog glasnika*, koji je izlazio od 1930. do 1941. godine

IZDAVAČ
Ministarstvo odbrane Republike Srbije, Univerzitet odbrane, Beograd, Srbija

ISSN 0042-8450
eISSN 2406-0720
Open Access
(CC BY-SA)

Adresa redakcije: Univerzitet odbrane, Medicinski fakultet Vojnomedicinske akademije, Centar za medicinske naučne informacije, Crnotravska 17, 11 040 Beograd, Srbija. Informacije o pretplati (tel.): +381 11 3608 997. E-mail (redakcija): **vsp@vma.mod.gov.rs**

Radove objavljene u „Vojnosanitetskom pregledu“ indeksiraju: Science Citation Index Expanded (SCIE), Journal Citation Reports/Science Edition, SCOPUS, Excerpta Medica (EMBASE), Google Scholar, EBSCO, Biomedicina Serbica, Srpski citatni indeks (SCIndeks), DOAJ. Sadržaje objavljuju Giornale di Medicine Militare i Revista de Medicina Militara. Prikaze originalnih radova i izvoda iz sadržaja objavljuje International Review of the Armed Forces Medical Services.

Časopis izlazi dvanaest puta godišnje. Pretplate: Žiro račun br. 840-19540845-28, poziv na broj 122742313338117. Za pretplatu iz inostranstva obratiti se službi pretplate na tel. +381 11 3608 997. Godišnja pretplata: 5 000 dinara za građane Srbije, 10 000 dinara za ustanove iz Srbije i 150 € za pretplatnike iz inostranstva. Kopiju uplatnice dostaviti na gornju adresu.

Štampa: Vojna štamparija, Beograd, Resavska 40b

CONTENTS / SADRŽAJ

July of this year marks 106 years since the birth of Rosalind Franklin (July 25, 1920–April 16, 1958). In the very same issue of the journal Nature in which James Watson and Francis Crick published their double-helix model of DNA on April 25, 1953, Rosalind Franklin and her student Raymond Gosling published their X-ray diffraction photograph of B-DNA. Rosalind Franklin's contribution to elucidating the DNA structure is widely recognized today, although this was not the case at the time. She passed away from ovarian cancer at the age of 37. Rosalind Franklin's research was continued by her colleagues, who remarked that she "turned everything she touched into something extraordinary," inspiring them to uphold the same high scientific standards.

U julu ove godine navršava se 106 godina od rođenja Rozalind Frenklin (25.07.1920–16.04.1958). U istom broju časopisa Nature u kome su 25. aprila 1953. godine Džejms Vatson i Frensis Krik objavili model dvostruke spirale DNK, Rozalind Frenklin i njen učenik Rejmond Gosling objavili su rendgensku difrakcionu fotografiju B-DNK. Doprinos Rozalind Frenklin u razjašnjenju strukture DNK danas je priznat, što nije bio slučaj u tim godinama. Preminula je od raka jajnika u 37. godini života. Istraživanja Rozalind Frenklin nastavili su njeni saradnici koji su govorili da je „sve čega bi se dotakla pretvarala u nešto izuzetno" inspirišući ih da dostignu iste visoke naučne standarde.

CURRENT TOPIC

UDC: 616-002.5:615.281]:616.36
DOI: https://doi.org/10.2298/VSP260329039J

Antituberculosis drug-induced liver injury: current concepts in pathogenesis, diagnosis, and management

Oštećenje jetre izazvano antituberkuloticima: aktuelni koncepti u patogenezi, dijagnozi i lečenju

Jovan Javorac*†‡, Ana Milenković*†, Emilija Vujičić*†, Dragica Kovačević*†, Tijana Rudić Vranić§, Darko Mikić‖¶, Dejan Živanović*‡**

***University of Novi Sad, Faculty of Medicine, Novi Sad, Serbia; †Institute for Pulmonary Diseases of Vojvodina, Sremska Kamenica, Serbia; ‡College of Vocational Studies for the Education of Preschool Teachers and Sports Trainers, Subotica, Serbia; §Galen Pharm Pharmacies, Belgrade, Serbia; ‖University of Defence, Faculty of Medicine of the Military Medical Academy, Belgrade, Serbia; ¶Military Medical Academy, Pathology and Forensic Medicine Institute, Belgrade, Serbia; **Alfa BK University, Belgrade, Serbia**

Abstract

Antituberculosis drug-induced liver injury (ATB-DILI) is one of the most significant adverse effects of first-line tuberculosis therapy and a leading cause of treatment discontinuation. Hepatotoxicity associated with isoniazid, rifampicin, and pyrazinamide may substantially impair treatment adherence, prolong treatment duration, and, in severe cases, lead to acute liver failure. This paper provides an overview of contemporary trends in the epidemiology, pathogenesis, diagnosis, and management of ATB-DILI, with particular emphasis on clinically relevant aspects and ongoing controversies. The underlying mechanisms of ATB-DILI origin involve complex interactions between drug metabolism, genetic susceptibility, oxidative stress, mitochondrial dysfunction, and immune-mediated injury, with emerging evidence suggesting a potential role of ferroptosis. Diagnosis relies on biochemical and clinical criteria, the exclusion of alternative causes, and causality assessment tools, such as the Roussel Uclaf Causality Assessment Method – RUCAM scale. Early recognition and timely discontinuation of hepatotoxic agents are of paramount importance, while optimal strategies for treatment reintroduction and the role of hepatoprotective agents are still not fully defined. A better understanding of risk factors and mechanisms, alongside the development of predictive biomarkers and pharmacogenetic approaches, may enable more individualized and safer tuberculosis treatment.

Keywords:
antitubercular agents; chemical and drug-induced liver injury; diagnosis; drug-related side effects and adverse reactions; tuberculosis.

Apstrakt

Oštećenje jetre izazvano antituberkuloticima (*antituberculosis drug-induced liver injury* – ATB-DILI) jedan je od najznačajnijih neželjenih efekata terapije prve linije tuberkuloze i jedan od glavnih uzroka prekida lečenja. Hepatotoksičnost povezana sa izoniazidom, rifampicinom i pirazinamidom može značajno narušiti adherenciju, produžiti trajanje terapije i, u težim slučajevima, dovesti do akutne insuficijencije jetre. Ovaj rad pruža pregled savremenih trendova u epidemiologiji, patogenezi, dijagnostici i lečenju ATB-DILI, sa posebnim naglaskom na klinički bitne aspekte i aktuelne dileme. Osnovni mehanizmi nastanka ATB-DILI uključuju složene interakcije između metabolizma lekova, genetske predispozicije, oksidativnog stresa, mitohondrijalne disfunkcije i imunski posredovanog oštećenja, sa novim dokazima koji ukazuju na potencijalnu ulogu feroptoze. Dijagnoza se zasniva na biohemijskim i kliničkim kriterijumima, isključenju drugih uzroka i alatima za procenu uzročnosti, kao što je Roussel Uclaf Causality Assessment Method – RUCAM skala. Rano prepoznavanje i pravovremeni prekid terapije hepatotoksičnim lekovima od ključnog su značaja, dok optimalne strategije ponovnog uvođenja terapije i uloga hepatoprotektivnih agenasa još uvek nisu definisane u potpunosti. Bolje razumevanje faktora rizika i mehanizama, uz razvoj prediktivnih biomarkera i farmakogenetskih pristupa, može omogućiti personalizovanije i bezbednije lečenje tuberkuloze.

Ključne reči:
antituberkulotici; jetra, oštećenje, hemijsko i lekovima izazvano; dijagnoza; lekovi, neželjeni efekti i neželjene reakcije; tuberkuloza.

Correspondence to: Jovan Javorac, Institute for Pulmonary Diseases of Vojvodina, Put dr Goldmana 4, 21 204 Sremska Kamenica, Serbia. E-mail: jovan.javorac@mf.uns.ac.rs

Introduction

Although tuberculosis (TB) is sometimes considered a forgotten disease, it remains a major global public health problem. According to the recent estimates [1], over 10 million people develop TB annually, and the disease continues to be a leading cause of death from a single infectious agent. Major factors contributing to inadequate disease control include human immunodeficiency virus (HIV) infection, poverty, malnutrition, diabetes mellitus, smoking, alcohol misuse, as well as treatment interruptions related to adverse effects of the therapy [2].

Although the World Health Organization (WHO) has recently recommended a shortened 4-month rifapentine-based regimen for the treatment of drug-susceptible TB in selected patient populations [3], the standard 6-month regimen remains the cornerstone of therapy in most settings. This regimen includes a combination of isoniazid (INH), rifampicin (RMP), pyrazinamide (PZA), and ethambutol (EMB) during the initial 2 months of treatment, followed by INH and RMP for an additional 4 months during the continuation phase. Despite high treatment success rates [1], the effectiveness of therapy may be compromised by adverse drug reactions, which can contribute to treatment failure, increased morbidity and mortality, and the development of drug resistance [4, 5].

Among these, hepatotoxicity, referred to as antituberculosis (ATB) drug-induced liver injury (LI) – DILI (ATB-DILI), is one of the most common and clinically significant complications. Hepatotoxicity associated with first-line agents, mainly INH, RMP, and PZA, may lead to treatment interruption, reduced adherence, prolonged therapy, and, in severe cases, liver failure (LF) [6]. Although substantial progress has been made in understanding the mechanisms, risk factors (RF), and clinical management of ATB-DILI, important uncertainties remain, particularly regarding early prediction, optimal monitoring strategies, and safe reintroduction of therapy. This paper provides an overview of current concepts in the pathogenesis, diagnosis, and management of ATB-DILI, with emphasis on clinically relevant aspects and ongoing controversies.

Definition of antituberculosis drug-induced liver injury

According to the clinical practice guidelines of the European Association for the Study of the Liver, DILI, regardless of the causative agent, is defined by one of the following laboratory criteria: elevation of alanine aminotransferase (ALT) ≥ 5 times the upper limit of normal (× ULN), elevation of alkaline phosphatase (ALP) ≥ 2 × ULN, or combined increase in ALT ≥ 3 × ULN and total bilirubin (TBIL) ≥ 2 × ULN in the absence of alternative causes of LI [7]. These biochemical criteria are widely used in clinical research and practice to standardize the diagnosis of DILI.

A wide range of medications may induce DILI. While acetaminophen is the leading cause of intrinsic hepatotoxicity, ATB drugs rank among the most frequent causes of idiosyncratic DILI on a global scale [8, 9]. In addition to these drugs, numerous other medications are commonly associated with DILI, including antibiotics (e.g., amoxicillin-clavulanate), antiepileptic drugs (e.g., valproate), amiodarone, nonsteroidal anti-inflammatory drugs, statins, and herbal and dietary supplements [10].

ATB-DILI represents one of the most clinically significant complications of TB treatment because it may lead to treatment interruption, modification of therapeutic regimens, prolongation of therapy duration, and, in severe cases, increased morbidity and mortality due to acute LF [6, 11]. Clinical manifestations range from asymptomatic elevations of liver enzymes, which occur in approximately 50–75% of patients, to symptomatic hepatitis characterized by nausea, vomiting, jaundice, and coagulopathy, and in rare cases, progression to acute LF [12]. Because hepatotoxicity most frequently occurs during the intensive phase of therapy, careful clinical and biochemical monitoring is recommended during the early stages of treatment, particularly in patients with known RF for LI, in order to allow early detection of hepatotoxicity and prevent progression to severe liver damage [13].

Among first-line ATB drugs, INH, RMP, and PZA are most commonly associated with hepatotoxicity, particularly when used in combination. In contrast, EMB and streptomycin have minimal hepatotoxic potential [14]. When administered as monotherapy (such as in the treatment of latent TB infection), INH is the most frequent cause of hepatotoxic reactions, whereas PZA is generally considered the most hepatotoxic drug in a dose-dependent manner. RMP rarely causes severe hepatocellular injury (HCI) on its own but may enhance hepatotoxicity through interactions with other ATB drugs [15]. With the increasing prevalence of drug-resistant TB, hepatotoxicity associated with second-line ATB drugs has also gained clinical importance. Among these agents, bedaquiline and pretomanid have been associated with a higher risk of LI, while delamanid and respiratory fluoroquinolones appear to have a more favorable hepatic safety profile [5, 16, 17].

Classification of antituberculosis drug-induced liver injury

DILI can be classified according to pathogenesis and predictability into intrinsic and idiosyncratic forms [10]. Intrinsic (direct) DILI represents a predictable and dose-dependent type of hepatotoxicity that occurs in most individuals once the concentration of the offending drug or its toxic metabolite in hepatocytes exceeds a certain threshold. This type of LI usually has a short latency period, ranging from several hours to a few days, and can be reproduced in experimental models. The underlying mechanism typically involves direct toxicity of reactive metabolites leading to oxidative stress (OS), mitochondrial dysfunction, and hepatocyte necrosis [18]. The prototypical example of intrinsic DILI is acetaminophen-induced hepatotoxicity, which represents the most common cause of acute drug-related LF in many developed countries [7]. In contrast, idiosyncratic DILI is unpredictable and generally

not strictly dose-dependent. It occurs in a relatively small proportion of exposed individuals and usually develops after a longer latency period, typically several days to weeks after drug exposure. Idiosyncratic hepatotoxicity is often associated with genetic susceptibility, interindividual differences in drug metabolism, or immune-mediated mechanisms. In such cases, reactive drug metabolites may bind to hepatocellular proteins, forming hapten-protein complexes that trigger immune responses directed against liver tissue [19]. In the context of ATB therapy (ATBT), most cases of LI are considered idiosyncratic because they occur in only a minority of treated patients and are frequently associated with genetic polymorphisms affecting drug metabolism. Nevertheless, some ATB drugs may also exhibit elements of intrinsic hepatotoxicity due to the formation of reactive metabolites capable of directly damaging hepatocytes, as observed with INH and PZA [12].

Another important classification of ATB-DILI is based on the biochemical pattern of LI, determined according to the dominant elevation of liver enzymes. The biochemical pattern of ATB-DILI is commonly classified using the R-ratio, calculated as serum (ALT/ULN) / serum (ALP/ULN). Based on this value, LI can be categorized as hepatocellular ($R \geq 5$), cholestatic ($R \leq 2$), or mixed ($2 < R < 5$) [19].

Epidemiology

The reported incidence of ATB-DILI varies considerably depending on the diagnostic criteria used and the geographic region studied, which may influence both the prevalence of TB and genetic susceptibility to hepatotoxicity. Overall, ATB-DILI has been reported in approximately 2–28% of patients receiving ATBT [5, 14]. A recent large meta-analysis including over 100,000 patients reported a pooled incidence of approximately 11.5%, with an increasing temporal trend worldwide [20]. The incidence of ATB-DILI demonstrates significant geographic variability, being lower in low TB-burden regions such as Western countries [21, 22] and higher in endemic areas, particularly within the African [6, 23] and the Asia–Pacific regions [24–26]. Although most patients with ATB-DILI experience complete clinical and biochemical recovery following withdrawal or modification of therapy, severe LI may develop in a subset of cases requiring hospitalization. In such situations, progression to acute LF may occur and may necessitate liver transplantation, with reported mortality rates ranging from 10% to 35% in severe cases [11].

The prognosis of ATB-DILI largely depends on the pattern of LI. HCI is generally associated with the highest risk of severe outcomes, whereas mixed patterns tend to have a more favorable prognosis [7].

Numerous studies have identified several RF associated with the development of ATB-DILI. These include older age, female sex, alcohol consumption, HIV infection, chronic viral hepatitis (hepatitis B or hepatitis C virus), pre-existing chronic liver disease, malnutrition and hypoproteinemia, anemia, as well as genetic polymorphisms in enzymes involved in the metabolism of ATB drugs, particularly variants affecting N-acetyltransferase (NAT) - 2 activity [26–29].

Mechanisms of hepatotoxicity

The hepatotoxic effects of ATB drugs arise from complex interactions between drug metabolism, genetic susceptibility, OS, and immune-mediated mechanisms. Among first-line ATB agents, INH, RMP, and PZA, in other words, their integrated mechanisms play the most important role in the development of ATB-DILI [5, 15, 18, 30, 31] (Figure 1).

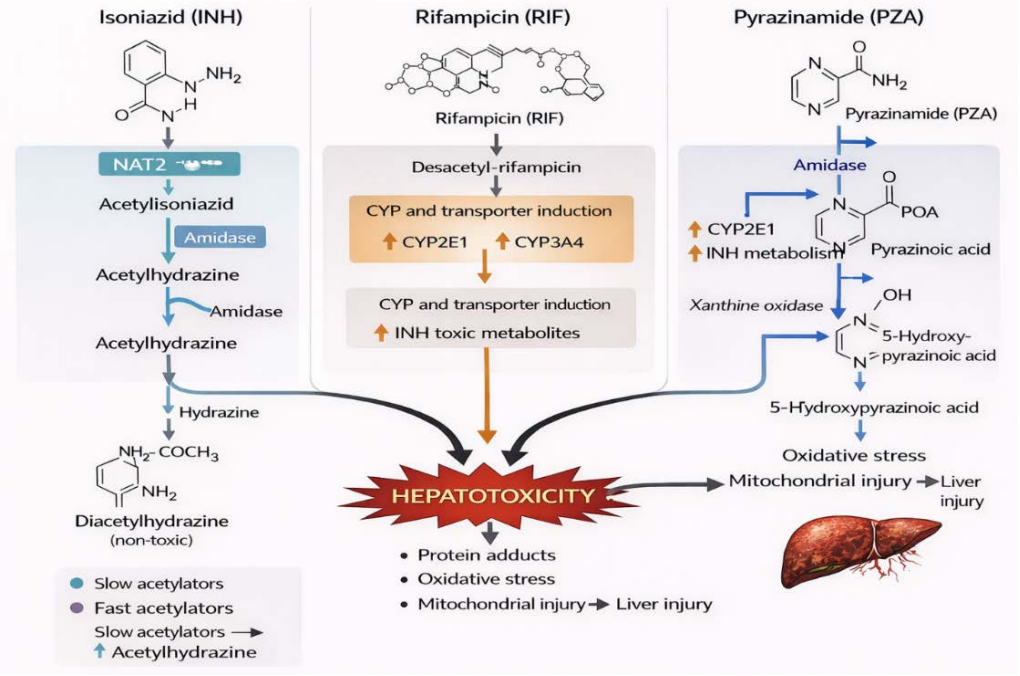

Fig. 1 – Integrated mechanism of antituberculosis drug-induced liver injury (ATB-DILI) development. CYP – cytochrome P450.

***Note*: Illustration generated by the author based on the cited papers [5, 15, 18, 30, 31].**

Isoniazid

INH undergoes extensive hepatic metabolism, primarily through acetylation catalyzed by the enzyme NAT2. In this process, INH is converted into acetylisoniazid, which is subsequently hydrolyzed to isonicotinic acid and acetylhydrazine. Acetylhydrazine represents a key intermediate metabolite that can follow two metabolic pathways. In the detoxification pathway, acetylhydrazine is further acetylated by NAT2 to form diacetylhydrazine, a relatively non-toxic metabolite. Alternatively, acetylhydrazine may be oxidized by cytochrome P450 (CYP) enzymes, particularly CYP2E1, yielding reactive, hepatotoxic intermediates that can induce OS, mitochondrial dysfunction, and hepatocyte necrosis. Additionally, a portion of INH can be directly hydrolyzed to hydrazine, another hepatotoxic metabolite that may also be oxidized by CYP2E1. Reactive metabolites generated during these processes may be detoxified through conjugation with glutathione, primarily mediated by glutathione-S-transferase [5].

Genetic polymorphisms affecting NAT2 activity play a crucial role in determining susceptibility to INH-induced hepatotoxicity [32]. Individuals are commonly classified as rapid, intermediate, or slow acetylators. In slow acetylators, reduced metabolic capacity leads to higher circulating levels of INH and its toxic metabolites, thereby increasing the risk of adverse effects, including hepatotoxicity and peripheral neuropathy [33, 34]. Several studies and meta-analyses have demonstrated a significantly increased risk of INH-induced LI among individuals with slow NAT2 acetylator status [4]. In addition to NAT2, other genetic factors may contribute to susceptibility to ATB-DILI. The *GSTM1* homozygous null genotype, associated with reduced detoxification capacity, and the *CYP2E1* c1/c1 genotype, linked to increased formation of hepatotoxic metabolites, have both been associated with an increased risk of LI [5, 25]. Furthermore, emerging pharmacogenetic studies highlight substantial interindividual and interethnic variability in the distribution of these genetic variants, which may partly explain differences in ATB-DILI incidence across populations [35]. Slow acetylator genotypes, more common in European, South American Indigenous, and Middle Eastern populations, are associated with increased hepatotoxicity risk [24, 35].

Rifampicin

RMP rarely causes severe HCI when administered alone; however, it can significantly increase the risk of hepatotoxicity during combination ATBT. RMP is a potent inducer of hepatic drug-metabolizing enzymes through activation of the pregnane X receptor, which upregulates the expression of multiple CYP enzymes and transport proteins involved in bile acid metabolism. This induction accelerates the metabolism of co-administered drugs, particularly INH, thereby promoting the formation of hepatotoxic metabolites [18].

In addition, RMP may interfere with hepatobiliary transport mechanisms, potentially leading to cholestatic or mixed patterns of LI [36]. Genetic variability in hepatic transporters and metabolic enzymes may further influence intrahepatic concentrations of RMP and modulate individual susceptibility to hepatotoxicity [5].

Pyrazinamide

PZA is considered one of the most hepatotoxic first-line ATB drugs, and its hepatotoxicity appears to be dose-dependent. In the liver, PZA is metabolized by microsomal amidase to pyrazinoic acid, which is subsequently oxidized by xanthine oxidase to form 5-hydroxypyrazinoic acid. These metabolites may induce mitochondrial dysfunction, OS, and direct HCI, leading predominantly to a hepatocellular pattern of liver damage. Because of its hepatotoxic potential, PZA is generally administered only during the initial intensive phase of TB treatment [30].

Emerging mechanisms

Despite significant advances in understanding ATB-DILI, the precise mechanisms of hepatotoxicity remain incompletely elucidated. Recent experimental studies have suggested a potential role of ferroptosis, a form of programmed cell death characterized by iron-dependent lipid peroxidation [31]. This process appears to be regulated by the HIF-1α/SLC7A11/GPx4 signaling pathway. Suppression of the cystine transporter SLC7A11 results in depletion of intracellular glutathione, impairing the activity of glutathione peroxidase 4, a key enzyme responsible for neutralizing reactive lipid species. As a consequence, lipid peroxidation accumulates and promotes hepatocyte injury. Elevated intracellular iron levels may further amplify OS and enhance the susceptibility of hepatocytes to ATB drug toxicity [36].

Overall, pharmacogenetic research in ATB-DILI is evolving from single-gene associations toward a more integrated, multi-gene framework that includes metabolic enzymes, detoxification pathways, and transport systems. In addition to genetic polymorphisms affecting drug metabolism, variants in hepatic transporters, including *ABCB11*, which encodes the bile salt export pump, have also been implicated in susceptibility to ATB-DILI, suggesting that impaired bile acid transport may contribute to cholestatic or mixed patterns of LI [37]. Although most evidence still derives from studies on INH metabolism, emerging data indicate that genetic variability may also influence hepatotoxicity associated with other ATB drugs [32, 38]. However, compared to INH, these findings remain less consistent and are often population-specific, highlighting the need for further validation in large, prospective studies. While integrating multiple genetic markers may improve risk stratification and support the concept of individualized TB therapy, routine pharmacogenetic testing has not yet been widely implemented due to limited availability, cost, and lack of standardization [39].

Diagnostic criteria for antituberculosis drug-induced liver injury

The diagnosis of ATB-DILI is based on a combination of biochemical evidence of LI, clinical assessment, and evaluation of the causal relationship between LI and exposure to ATB drugs [7, 40–42] (Figure 2). Because no single diagnostic test exists, ATB-DILI remains a diagnosis of exclusion.

Biochemical criteria for drug-induced liver injury

Initial laboratory evaluation of suspected ATB-DILI should include measurement of ALT, aspartate aminotransferase (AST), ALP, gamma-glutamyltransferase (GGT), total and direct bilirubin, and coagulation parameters such as prothrombin time or international normalized ratio (INR) [7, 40]. As previously described, according to established clinical guidelines, DILI is defined by specific biochemical thresholds involving elevations in ALT, ALP, and TBIL [7]. Other proposed biochemical criteria for DILI [3, 7, 40, 41, 43] are shown in Table 1.

It is important to distinguish mild asymptomatic elevations of liver enzymes from clinically significant hepatotoxicity, as transient abnormalities in liver function tests may occur during ATBT without fulfilling diagnostic criteria for DILI [44]. Persistent elevation of TBIL and ALP beyond 2 months after the onset of DILI is considered indicative of chronic DILI [7].

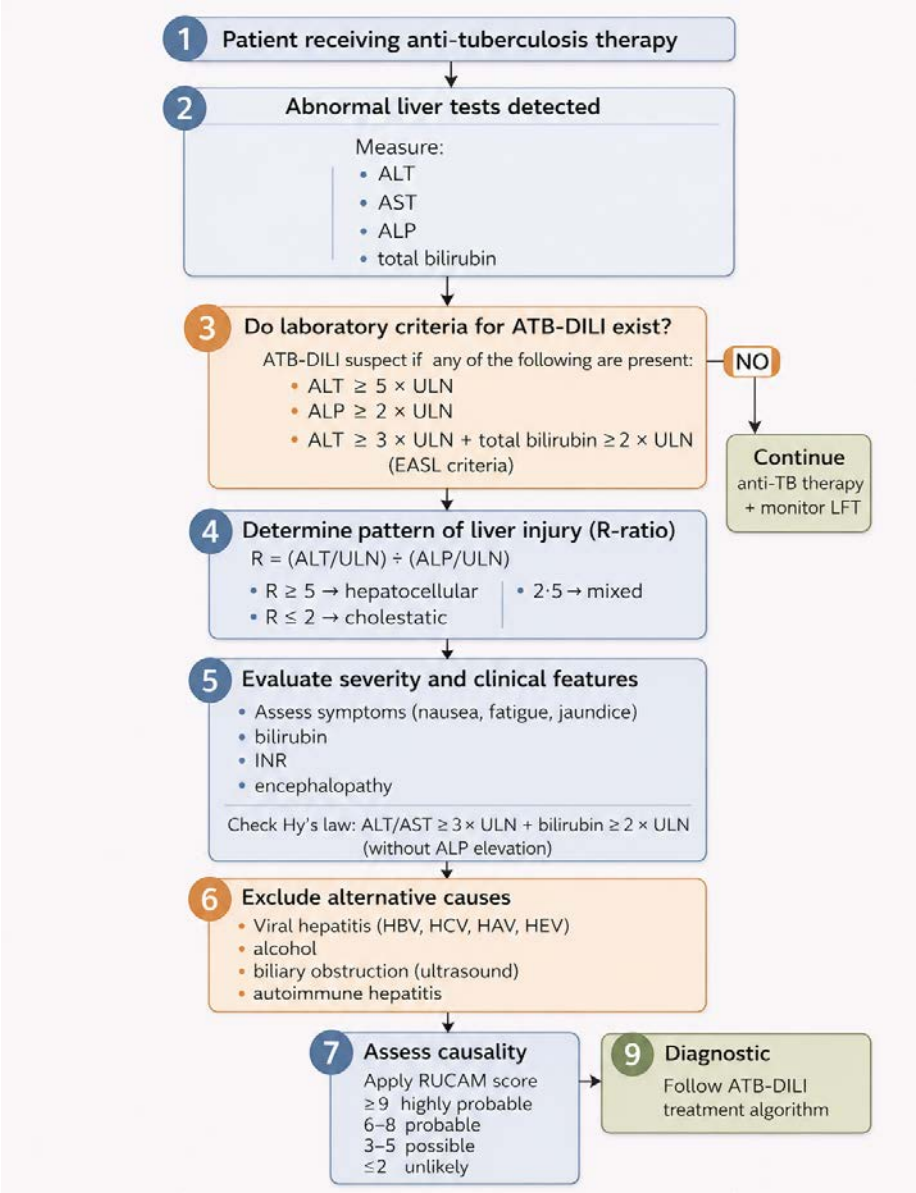

Fig. 2 – Proposed diagnostic algorithm for suspected anti-tuberculosis drug-induced liver injury (ATB-DILI). ALT – alanine aminotransferase; AST – aspartate aminotransferase; ALP – alkaline phosphatase; ULN – upper limits of normal; EASL – European Association for the Study of the Liver; TB – tuberculosis; LFT – liver function tests; INR – international normalized ratio; HBV – hepatitis B virus; HCV – hepatitis C virus; HAV – hepatitis A virus; HEV – hepatitis E virus; RUCAM – Roussel-Uclaf Causality Assessment Method.
***Note*: Illustration generated by the author based on the cited papers [7, 40, 41, 42].**

Table 1

Diagnostic criteria and treatment interruption thresholds for drug-induced liver injury (DILI) in patients receiving antituberculosis therapy

Guideline/Source	Diagnostic criteria for ATB-DILI	Criteria for interruption of anti-TB therapy	Key notes
European Association for the Study of the Liver [7]	ALT ≥ 5 × ULN; or ALP ≥ 2 × ULN; or ALT ≥ 3 × ULN + TBIL ≥ 2 × ULN	not specifically defined for TB therapy	widely accepted diagnostic criteria for DILI used in clinical research and hepatology practice
American Association for the Study of Liver Diseases [41]	same biochemical criteria as EASL	not specifically defined for TB therapy	emphasizes causality assessment, Hy's law, and exclusion of alternative causes
Chinese Medical Association Tuberculosis Branch [40]	ALT ≥ 3 × ULN and/or TBIL ≥ 2 × ULN; or simultaneous elevation of AST, ALP, and TBIL with ≥ 2 × ULN for at least one parameter	not TB-specific	recommends broader biochemical testing including GGT and coagulation parameters
ATS/CDC/IDSA [43]	not primarily focused on defining DILI	ALT ≥ 5 × ULN without symptoms or ALT ≥ 3 × ULN with symptoms	mild DILI – ALT < 5 × ULN moderate DILI – ALT 5–10 × ULN severe DILI – ALT ≥ 10 × ULN
WHO [3]	not specifically defining DILI	ALT ≥ 5 × ULN (asymptomatic) or ≥ 3 × ULN (symptomatic)	global TB management guidance largely consistent with ATS recommendations

ATB-DILI – antituberculosis drug-induced liver injury; TB – tuberculosis; ALT – alanine aminotransferase; ULN – upper limits of normal; ALP – alkaline phosphatase; TBIL – total bilirubin; EASL – European Association for the Study of the Liver; GGT – gamma-glutamyl transferase; ATS – American Thoracic Society; CDC – Center for Disease Control and Prevention; IDSA – Infectious Diseases Society of America; WHO – World Health Organization.

After confirming biochemical LI, the pattern of injury should be determined using the R-ratio. As previously mentioned, based on this value, three patterns of ATB-DILI are recognized: HCI (R ≥ 5), cholestatic injury (R ≤ 2), or mixed injury (2 < R < 5) [19]. The hepatocellular pattern is characterized by a predominant elevation of ALT with relatively normal or mildly elevated ALP levels and reflects primary hepatocyte injury. The cholestatic pattern is characterized by a predominant increase in ALP, often accompanied by elevated bilirubin levels, indicating impairment of hepatobiliary transport and bile canalicular injury. The mixed pattern represents an intermediate form in which both transaminases and ALP are significantly elevated [5]. In the context of ATB-DILI, HCI is most commonly associated with INH and PZA, whereas RMP more frequently produces cholestatic or mixed patterns of LI [19].

Particular clinical importance is attributed to the concept known as Hy's law, which describes the presence of HCI (ALT or AST ≥ 3 × ULN) accompanied by elevation of TBIL ≥ 2 × ULN in the absence of significant cholestasis. This pattern represents a strong predictor of severe DILI and is associated with an increased risk of acute LF and mortality [41].

Diagnostic criteria and treatment interruption thresholds for DILI in patients receiving ATBT [3, 7, 40, 41, 43] are shown in Table 1.

Clinical assessment

In addition to biochemical criteria, clinical evaluation plays an important role in the assessment of suspected ATB-DILI. Symptoms of hepatotoxicity may include fatigue, anorexia, nausea, vomiting, right upper quadrant abdominal pain, dark urine, and jaundice. However, a substantial proportion of patients may remain completely asymptomatic, which underscores the importance of routine monitoring of liver biochemical parameters during ATBT [14]. In severe cases, ATB-DILI may progress to acute LF characterized by coagulopathy and varying degrees of hepatic encephalopathy [15].

Assessment of severity

The severity of ATB-DILI may be classified according to biochemical abnormalities and clinical manifestations [44], ranging from mild liver enzyme elevation to acute LF characterized by coagulopathy and hepatic encephalopathy, as shown in Table 2. Severe forms of ATB-DILI may require hospitalization and, in rare cases, liver transplantation.

Severity classification of ATB-DILI that has been modified from Yu et al. [44] is shown in Table 2.

Causality assessment

Once DILI has been confirmed either biochemically or in combination with clinical findings, the next step is to evaluate causality, specifically, determining whether the observed LI is likely related to ATBT. The most widely used tool for this purpose is the Roussel-Uclaf Causality Assessment Method (RUCAM) [42]. RUCAM evaluates several parameters, including the temporal relationship between drug

Table 2

Severity classification of ATB-DILI (adapted from Yu et al. [44])

Liver injury, severity	Grade	Laboratory criteria	Clinical manifestations
None	0	no elevation of ALT or ALP; normal TBIL and INR	none
Mild	1	elevated ALT and/or ALP with TBIL < 2.5 mg/dL and INR < 1.5	usually mild or nonspecific symptoms such as fatigue, nausea, malaise, pruritus, or right upper quadrant discomfort
Moderate	2	elevated ALT and/or ALP with TBIL ≥ 2.5 mg/dL or INR ≥ 1.5 (without hepatic failure)	more pronounced symptoms of liver injury; jaundice may occur
Severe	3	elevated ALT and/or ALP with TBIL ≥ 2.5 mg/dL and/or INR ≥ 1.5 requiring hospitalization	clinically significant liver injury requiring hospital admission
Acute liver failure	4	marked elevation of liver enzymes with TBIL ≥ 2.5 mg/dL and evidence of hepatic failure (e.g., INR ≥ 1.5 with hepatic encephalopathy, ascites, or other signs of hepatic decompensation)	acute liver failure with encephalopathy, coagulopathy, or multi-organ involvement
Fatal outcome	5	liver failure leading to death or need for liver transplantation	death or liver transplantation

ATB-DILI – antituberculosis drug-induced liver injury; ALT – alanine aminotransferase; ALP – alkaline phosphatase; TBIL – total bilirubin; INR – international normalized ratio.

exposure and onset of LI, the course of liver enzyme changes after drug withdrawal (dechallenge), the presence of RF such as alcohol consumption or older age, exclusion of alternative causes of LI, and the known hepatotoxic potential of the suspected drug. Based on the total score, causality is categorized as follows: highly probable (≥ 9 points), probable (6–8 points), possible (3–5 points), unlikely (1–2 points), and excluded (≤ 0 points) [7]. Despite its widespread use, RUCAM has several important limitations. Its application may be challenging in the setting of polypharmacy, as it requires separate assessment for each drug and may be affected by incomplete clinical data and interobserver variability [42]. Furthermore, the hepatotoxic risk of newer biologics and herbal products may be underestimated due to inadequate labeling and limited safety data in the RUCAM database [45].

Exclusion of alternative causes

Because DILI is fundamentally a diagnosis of exclusion, other causes of LI must be systematically ruled out in order to confirm ATB-DILI. These include viral infections such as hepatitis B and C, HIV infection (in immunocompromised Epstein-Barr virus, cytomegalovirus, and herpes simplex virus), autoimmune liver diseases, alcohol-related liver disease, biliary obstruction, and exposure to other hepatotoxic medications (acetaminophen, statins) or herbal supplements [43]. Abdominal ultrasound is commonly performed in patients with suspected ATB-DILI to exclude structural liver disease or biliary obstruction [6]. However, imaging findings are typically nonspecific. Liver biopsy is generally not required for the diagnosis of ATB-DILI, but may be considered in selected cases when diagnostic uncertainty persists or when alternative etiologies such as autoimmune hepatitis are suspected [15].

Rechallenge

Re-exposure to the suspected drug (rechallenge) represents the strongest evidence for causality in ATB-DILI. A positive rechallenge is typically defined as a recurrent elevation of aminotransferases, usually ALT > 3 × ULN, following reintroduction of the suspected drug. Rechallenge should be undertaken with caution because recurrent LI may occur more rapidly and may be more severe than the initial episode [7]. However, in clinical practice, rechallenge is often avoided due to safety concerns, particularly in cases of severe initial hepatotoxicity or when drug labeling contraindicates re-exposure, as is the case with PZA [46].

Management of antituberculosis drug-induced liver injury

Management of ATB-DILI primarily involves prompt recognition of hepatotoxicity, temporary discontinuation of hepatotoxic drugs, and careful reintroduction of therapy once liver function improves. At the same time, clinicians must balance the risk of worsening LI with the need to maintain effective treatment of TB.

Discontinuation of antituberculosis drugs

During the initial phase of TB treatment, approximately 20% of patients may develop asymptomatic elevations of aminotransferases. In asymptomatic individuals with ALT levels < 5 × ULN, discontinuation of ATBT is generally not

required, although close clinical and biochemical monitoring is recommended [43]. However, treatment interruption is indicated in the following situations [3, 7, 40, 41, 43], as shown in Table 1: ALT ≥ 3 × ULN in the presence of symptoms suggestive of hepatotoxicity, or ALT ≥ 5 × ULN in asymptomatic patients, or significant elevation of bilirubin and/or ALP. In such cases, all potentially hepatotoxic ATB drugs should be discontinued. Following drug withdrawal, the majority of patients show gradual improvement in liver function, and most recover completely [25, 47]. Nevertheless, a small proportion of patients may develop chronic DILI or progress to acute LF [28].

Reintroduction of antituberculosis therapy

Once liver function improves, ATBT can usually be safely reintroduced in approximately 80–90% of patients [48]. Reintroduction is typically considered when serum transaminase levels decrease to < 2 × ULN, bilirubin levels normalize, and clinical symptoms resolve [49]. Drugs are generally reintroduced sequentially, one at a time, with close monitoring of liver enzymes in order to identify the offending agent if hepatotoxicity recurs [25]. A commonly used approach is to reintroduce drugs over a period of approximately 7–10 days, starting with the least hepatotoxic agents [19]. While both strategies–full-dose initiation and gradual dose escalation from minimum to maximum dose–have been described, current evidence does not support a significant difference in safety between these approaches [50].

Several strategies for reintroduction of ATBT after ATB-DILI have been described, reflecting differences across international guidelines and the lack of a universally accepted approach. However, recommendations from the WHO [3] and the National Institute for Health and Care Excellence (NICE) [49] are broadly aligned, favoring a cautious, stepwise reintroduction strategy. This approach typically involves initiating treatment with less hepatotoxic agents, such as EMB, followed by sequential addition of RMP and INH with close biochemical monitoring, while PZA is often omitted, particularly after severe LI. In those cases, treatment duration is subsequently extended to at least 8–9 months [43, 46]. In contrast, other guidelines propose alternative strategies, including full-dose initiation or gradual dose escalation of individual drugs; however, available evidence does not demonstrate clear superiority of any single approach [50]. Therefore, in clinical practice, reintroduction is generally individualized, guided by the severity of LI, the importance of specific drugs for TB treatment, and careful monitoring of liver function. If hepatotoxicity recurs following reintroduction, the suspected drug should be permanently discontinued. The proposed algorithm for reintroduction of ATB drugs, proposed by NICE [49], is shown in Figure 3.

Alternative antituberculosis regimens

In patients requiring continued ATB treatment despite hepatotoxicity, alternative regimens may be considered. In such situations, hepatotoxic agents such as INH, RMP, and PZA may be temporarily replaced with lower hepatotoxic potential drugs, including EMB and streptomycin, often combined with respiratory fluoroquinolones such as levofloxacin or moxifloxacin [49]. These regimens are typically used as temporary treatment strategies until standard therapy can be safely resumed.

Adjunctive hepatoprotective therapy

The role of hepatoprotective agents in ATB-DILI remains controversial. Despite widespread clinical use, hepatoprotective agents remain controversial due to heterogeneity of studies, small sample sizes, and lack of high-quality randomized trials.

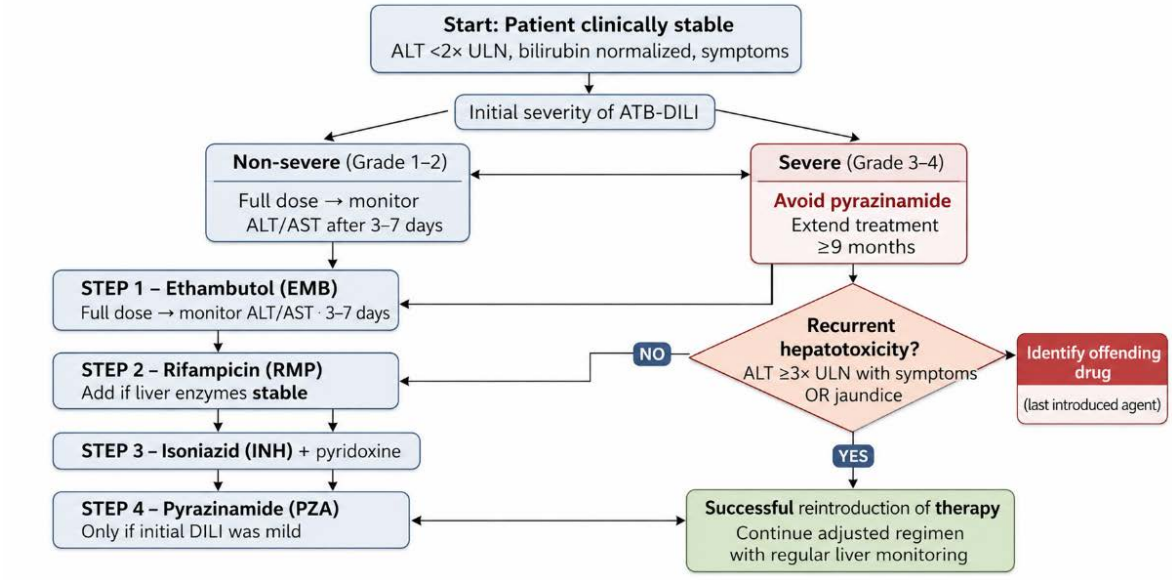

Fig. 3 – Algorithm for reintroduction of antituberculosis therapy after ATB-DILI. ALT – alanine aminotransferase; ULN – upper limits of normal; ATB-DILI – antituberculosis drug-induced liver injury; AST – aspartate aminotransferase.
***Note*: Illustration generated by the author based on the cited paper [49].**

N-acetylcysteine (NAC), which is well established in the treatment of acetaminophen-induced LI, has been investigated as a potential adjunctive therapy in non-acetaminophen DILI [51]. NAC acts as a precursor of glutathione and may exert antioxidant and anti-inflammatory effects. Some studies suggest that NAC may shorten recovery time and improve transplant-free survival in severe cases of DILI [52]; however, evidence remains limited, and its routine use in ATB-DILI has not yet been firmly established. Glucocorticoids are generally not recommended for routine treatment of ATB-DILI but may be considered in selected cases of immune-mediated DILI or when features of drug-induced autoimmune hepatitis are suspected [44].

Several hepatoprotective agents are frequently used in clinical practice as supportive therapy in patients with ATB-DILI, although robust evidence supporting their routine use remains limited [53]. These include compounds with antioxidant, anti-inflammatory, or membrane-stabilizing properties, such as silymarin, polyene phosphatidylcholine, glycyrrhizin derivatives, and S-adenosyl-L-methionine [40]. Some data suggest that these agents may contribute to faster normalization of liver enzymes and improvement of hepatic function [54]; however, current international guidelines do not recommend their routine use due to insufficient high-quality clinical evidence [40, 53].

Management of severe liver injury

In severe cases, ATB-DILI may progress to acute LF characterized by coagulopathy and hepatic encephalopathy. Such patients require intensive supportive care and evaluation for liver transplantation. Extracorporeal liver support techniques, including large-volume plasma exchange or albumin dialysis, may be used as temporary bridging therapies in selected cases [40]. Although liver transplantation can be life-saving in severe ATB-DILI, reported survival rates remain limited, largely due to the severity of the underlying LI at the time of transplantation [15].

Prevention

Prevention of ATB-DILI primarily involves identification and modification of known RF. One of the most important preventive strategies is avoidance of concomitant use of other hepatotoxic medications, including certain herbal and traditional medicinal products, with careful evaluation of the benefit–risk ratio of all prescribed drugs. In patients with chronic viral hepatitis, timely initiation of antiviral therapy should be considered when clinically indicated. Pharmacogenetic approaches have also been proposed as a strategy for preventing ATB-DILI. In particular, genotyping of the *NAT2* gene may allow dose adjustment of INH according to an individual's acetylator status, potentially reducing the risk of hepatotoxicity [40, 55, 56]. However, although promising, routine pharmacogenetic testing is expensive and has not yet been widely implemented in clinical practice [53].

The prophylactic use of hepatoprotective agents may be considered in patients at high risk for LI, although routine preventive use is generally not recommended due to limited evidence. Because the pathogenesis of ATB-DILI involves the formation of reactive metabolites and OS, antioxidant and anti-inflammatory agents such as NAC have been investigated as potential preventive strategies [15], but further clinical studies are required to confirm their effectiveness. Recent meta-analysis has also explored the potential protective effects of certain herbal preparations, including combinations containing *Curcuma longa* (turmeric) and *Tinospora cordifolia*, as well as polyherbal formulations [57]. Although some studies reported a reduction in the incidence of ATB-DILI [57], these findings are based on a limited number of heterogeneous trials, and additional well-designed studies are needed before such interventions can be recommended as part of standard clinical practice.

Conclusion

Antituberculosis drug-induced liver injury remains one of the most clinically significant complications of tuberculosis therapy. Although first-line antituberculosis drugs are highly effective, their hepatotoxic potential may lead to treatment interruption, prolonged therapy, and increased morbidity. Early recognition through regular monitoring of liver function tests, especially during the initial phase of treatment, is essential for preventing severe hepatic injury. Prompt discontinuation of hepatotoxic drugs and careful reintroduction of therapy after recovery remain the cornerstone of management.

Despite significant advances in understanding the mechanisms and clinical management of antituberculosis drug-induced liver injury, several important gaps remain. There is a lack of validated predictive biomarkers that could enable early identification of high-risk patients. In addition, pharmacogenetic approaches, although promising, are not yet routinely implemented in clinical practice due to limited availability and cost-effectiveness concerns. Future research should focus on the development of reliable risk prediction models integrating clinical, biochemical, and genetic factors, as well as on the standardization of treatment interruption and reintroduction protocols. Furthermore, emerging mechanisms such as ferroptosis may represent potential therapeutic targets, but their clinical relevance remains to be clarified.

Conflict of interest

The authors declare no conflict of interest.

R E F E R E N C E S

1. *World Health Organization*. Global tuberculosis report 2025 [Internet]. Geneva: WHO; 2025 [accessed 2026 Apr 4; cited 2026 May 14]. Available from: https://www.who.int/teams/global-programme-on-tuberculosis-and-lung-health/tb-reports/global-tuberculosis-report-2025
2. *Ilic M, Bjelic D, Javorac J, Tot Veres K, Zivanovic D, Kovačevic D,* et al. Knowledge is the most powerful tool in the fight against tuberculosis. J Infect Dev Ctries 2023; 17(8): 1099–106. DOI: 10.3855/jidc.18112.
3. *World Health Organization.* WHO operational handbook on tuberculosis. Module 4: treatment and care [Internet]. Geneva: WHO; 2025 [accessed 2026 Apr 30; cited 2026 May 14]. Available from: https://www.who.int/publications/i/item/9789240065116
4. *Mahajan R, Tyagi AK.* Pharmacogenomic insights into tuberculosis treatment shows the NAT2 genetic variants linked to hepatotoxicity risk: a systematic review and meta-analysis. BMC Genom Data 2024; 25(1): 103. DOI: 10.1186/s12863-024-01286-y.
5. *Gunter HM, Choshi P, Chimbetete T, Pedretti S, Lehloenya RJ, Sinxadi PZ,* et al. Genetic polymorphisms and adverse reactions to antituberculosis therapy. Pharmacogenomics 2025; 26(5–6): 207–21. DOI: 10.1080/14622416.2025.2509479.
6. *Ebata-Mboussa EF, Assiana DOE, Moyen N, Mouzinga FH, Bonsi ST, Elenga EB,* et al. Anti-tuberculosis drug-induced hepatotoxicity among patients undergoing tuberculosis treatment at the Antituberculosis Center of Brazzaville, Republic of Congo. IJID Reg 2026; 19: 100869. DOI: 10.1016/j.ijregi.2026.100869.
7. *European Association for the Study of the Liver.* EASL Clinical Practice Guidelines: Drug-induced liver injury. J Hepatol 2019; 70(6): 1222–61. DOI: 10.1016/j.jhep.2019.02.014.
8. *Devarbhavi H, Aithal G, Treeprasertsuk S, Takikawa H, Mao Y, Shasthry SM,* et al. Drug-induced liver injury: Asia Pacific Association of Study of Liver consensus guidelines. Hepatol Int 2021; 15(2): 258–82. DOI: 10.1007/s12072-021-10144-3.
9. *Andrade RJ, Chalasani N, Björnsson ES, Suzuki A, Kullak-Ublick GA, Watkins PB,* et al. Drug-induced liver injury. Nat Rev Dis Primers 2019; 5(1): 58. DOI: 10.1038/s41572-019-0105-0.
10. *Hosack T, Damry D, Biswas S*. Drug-induced liver injury: a comprehensive review. Ther Adv Gastroenterol 2023; 16: 17562848231163410. DOI: 10.1177/17562848231163410.
11. *Liu Q, Huang L, Yan H, Zong Z, Chen Z, Wu X,* et al. Clinical risk factors for moderate and severe antituberculosis drug-induced liver injury. Front Pharmacol 2024; 15: 1406454. DOI: 10.3389/fphar.2024.1406454.
12. *Yang R, Jin Z, Jin X, Li Y, Wang D, Chao J*. Advances in antituberculosis drug-induced liver injury: Molecular mechanisms, genetic insights, and emerging animal-free prediction models. Toxicology 2026; 520: 154359. DOI: 10.1016/j.tox.2025.154359.
13. *Patterson B, Abbara A, Collin S, Henderson M, Shehata M, Gorgui-Naguib H,* et al. Predicting drug-induced liver injury from antituberculous medications by early monitoring of liver tests. J Infect 2021; 82(2): 240–4. DOI: 10.1016/j.jinf.2020.09.038.
14. *Zhang S, Dong N, Wang L, Lu Y, Chen X*. Clinical Features of Anti-Tuberculosis Drug-Induced Liver Injury and Risk Factors for Severe Cases: A Retrospective Study in China. Infect Drug Resist 2025; 18: 2065–78. DOI: 10.2147/IDR.S519211.
15. *Kumar R, Kumar A, Kumar S*. Acute liver failure from antituberculosis drug-induced liver injury: An update. World J Hepatol 2025; 17(5): 106618. DOI: 10.4254/wjh.v17.i5.106618.
16. *He Q, Li Y, Liu S, Xue H, Xiang X, Wang T,* et al. Drug-induced liver injury associated with pretomanid, bedaquiline, and linezolid: Insights from FAERS database analysis. Br J Clin Pharmacol 2025; 91(3): 799–807. DOI: 10.1111/bcp.16318.
17. *Al Omar MFS, Alchikhsuliman IEM, Awad SM, Rajab N, Alawi YM, Ibrahim LYM,* et al. Hepatic Safety of Bedaquiline, Delamanid, and Pretomanid: A Systematic Review and Meta-analysis. Int J Mycobacteriol 2025; 14(4): 309–19. DOI: 10.4103/ijmy.ijmy_97_25.
18. *Batool Z, Wu Q, Weng G, Shen B.* A Review on Isoniazid and Rifampicin-Associated Hepatotoxicity: From Metabolism to Immunity. Arch Pharm (Weinheim) 2026; 359(3): e70204. DOI: 10.1002/ardp.70204.
19. *Rizzi A, Nucera E, Urbani S, Longhino D, Lohmeyer FM, Gambassi G,* et al. Antituberculosis drug-induced non-blistering systemic severe reactions: A 10-year (2012-2022) literature review. Asian Pac J Allergy Immunol 2023; 41(4): 273–91. DOI: 10.12932/AP-010423-1582.
20. *Wang N, Chen X, Hao Z, Guo J, Wang X, Zhu X,* et al. Incidence and Temporal Trend of Antituberculosis Drug-Induced Liver Injury: A Systematic Review and Meta-Analysis. J Trop Med 2022; 2022: 8266878. DOI: 10.1155/2022/8266878.
21. *Bessone F, Hernandez N, Medina-Caliz I, García-Cortés M, Schinoni MI, Mendizabal M,* et al. Drug-induced Liver Injury in Latin America: 10-year Experience of the Latin American DILI (LATINDILI) Network. Clin Gastroenterol Hepatol 2025; 23(1): 89–102. DOI: 10.1016/j.cgh.2024.06.030.
22. *Stephens C, Robles-Diaz M, Medina-Caliz I, Garcia-Cortes M, Ortega-Alonso A, Sanabria-Cabrera J,* et al. Comprehensive analysis and insights gained from long-term experience of the Spanish DILI Registry. J Hepatol 2021; 75(1): 86–97. DOI: 10.1016/j.jhep.2021.01.029.
23. *Petros Z, Tamirat A, Assefa W*. Antitubercular drug induced liver injury among tuberculosis patients in central Ethiopia. Sci Rep 2025; 15(1): 31309. DOI: 10.1038/s41598-025-15855-3.
24. *Gopalakrishna H, Chalasani N*. Drug-induced liver injury due to first-line antituberculosis medications in India: A major hindrance to achieve the goal of tuberculosis elimination. Indian J Gastroenterol 2025; 44(1): 4–7. DOI: 10.1007/s12664-024-01674-3.
25. *Pradhan RR, Yadav AK*. Incidence, Clinical Features, Associated Factors and Outcomes of Intensive Phase Antituberculosis Drug Induced Liver Injury Among Patients With Tuberculosis at a Tertiary Care Hospital in Nepal: A Descriptive Cross-Sectional Study. Health Sci Rep 2025; 8(4): e70686. DOI: 10.1002/hsr2.70686.
26. *Kumar R, Kumar A, Patel R, Prakash SS, Kumar S, Surya H,* et al. Incidence and risk factors of antituberculosis drug-induced liver injury in India: A systematic review and meta-analysis. Indian J Gastroenterol 2025; 44(1): 35–46. DOI: 10.1007/s12664-024-01643-w.
27. *Zhang F, Zhang F, Qin M, Li L*. Alcohol consumption as a risk factor for anti-tuberculosis drug induced liver injury: A systematic review and meta-analysis. Microb Pathog 2024; 196: 107003. DOI: 10.1016/j.micpath.2024.107003.
28. *Jiang F, Yan H, Liang L, Du J, Jin S, Yang S,* et al. Incidence and risk factors of anti-tuberculosis drug induced liver injury (DILI): Large cohort study involving 4652 Chinese adult tuberculosis patients. Liver Int 2021; 41(7): 1565–75. DOI: 10.1111/liv.14896.
29. *Abbara A, Chitty S, Roe JK, Ghani R, Collin SM, Ritchie A,* et al. Drug-induced liver injury from antituberculous treatment: a retrospective study from a large TB centre in the UK. BMC Infect Dis 2017; 17(1): 231. DOI: 10.1186/s12879-017-2330-z.
30. *Hussain Z, Zhu J, Ma X*. Metabolism and Hepatotoxicity of Pyrazinamide, an Antituberculosis Drug. Drug Metab Dispos 2021; 49(8): 679–82. DOI: 10.1124/dmd.121.000389.
31. *Liu Y, Chen W, Cen Y, Zhao X, Chen Z, Liang Y,* et al. Hepatocyte ferroptosis contributes to anti-tuberculosis drug-induced

liver injury: Involvement of the HIF-1α/SLC7A11/GPx4 axis. Chem Biol Interact 2023; 376: 110439. DOI: 10.1016/j.cbi.2023.110439.
32. *Ramachandran G, Swaminathan S*. Role of pharmacogenomics in the treatment of tuberculosis: a review. Pharmgenomics Pers Med 2012; 5: 89–98. DOI: 10.2147/PGPM.S15454.
33. *Tavkar V, Goyal A, Chopra V, Garg K, Sharma S*. The role of NAT2 genetic variants in anti-tuberculosis drug-induced liver injury (AT-DILI): a meta-analysis. Pharmacogenomics 2025; 26(17–8): 787–803. DOI: 10.1080/14622416.2026.2624364.
34. *Cheng J, Zhu J, Chen R, Zhang M, Han B, Zhu M,* et al. Genetic polymorphisms and anti-tuberculosis drug-induced liver injury: an umbrella review of the evidence. Int J Clin Pharm 2025; 47(3): 624–39. DOI: 10.1007/s11096-025-01880-9.
35. *Jaramillo-Valverde L, Horton MK, Poterico JA, Lanata CM, Guio H*. Pharmacogenetic Study of Anti-TB Drugs in the Native Ancestry Peruvian Population. Pharmacol Res Perspect 2025; 13(3): e70135. DOI: 10.1002/prp2.70135.
36. *Kim JH, Nam WS, Kim SJ, Kwon OK, Seung EJ, Jo JJ,* et al. Mechanism Investigation of Rifampicin-Induced Liver Injury Using Comparative Toxicoproteomics in Mice. Int J Mol Sci 2017; 18(7): 1417. DOI: 10.3390/ijms18071417.
37. *Cavaco MJ, Alcobia C, Oliveiros B, Mesquita LA, Carvalho A, Matos F,* et al. Clinical and Genetic Risk FJactors for Drug-Induced Liver Injury Associated with Anti-Tuberculosis Treatment-A Study from Patients of Portuguese Health Centers. J Pers Med 2022; 12(5): 790. DOI: 10.3390/jpm12050790.
38. *Barliana MI, Afifah NN, Yunivita V, Ruslami R*. Genetic polymorphism related to ethambutol outcomes and susceptibility to toxicity. Front Genet 2023; 14: 1118102. DOI: 10.3389/fgene.2023.1118102.
39. *Amorim G, Jaworski J, Yang J, Cordeiro-Santos M, Kritski AL, Figueiredo MC,* et al. Pharmacogenetics of tuberculosis treatment toxicity and effectiveness in a large Brazilian cohort. Pharmacogenet Genomics 2025; 35(2): 55–64. DOI: 10.1097/FPC.0000000000000552.
40. *Chinese Medical Association Tuberculosis Branch*. Guidelines for diagnosis and management of drug-induced liver injury caused by anti-tuberculosis drugs (2024 version). Zhonghua Jie He He Hu Xi Za Zhi 2024; 47(11): 1069–90. DOI: 10.3760/cma.j.cn112147-20240614-00338. [Chinese]
41. *Fontana RJ, Liou I, Reuben A, Suzuki A, Fiel MI, Lee W,* et al. AASLD practice guidance on drug, herbal, and dietary supplement-induced liver injury. Hepatology 2023; 77(3): 1036–65. DOI: 10.1002/hep.32689.
42. *Danan G, Teschke R*. RUCAM in drug and herb induced liver injury: the update. Int J Mol Sci 2015; 17(1): 14. DOI: 10.3390/ijms17010014.
43. *Nahid P, Dorman SE, Alipanah N, Barry PM, Brozek JL, Cattamanchi A,* et al. Official American Thoracic Society/Centers for Disease Control and Prevention/Infectious Diseases Society of America Clinical Practice Guidelines: Treatment of Drug-Susceptible Tuberculosis. Clin Infect Dis 2016; 63(7): e147–95. DOI: 10.1093/cid/ciw376.
44. *Yu YC, Mao YM, Chen CW, Chen JJ, Chen J, Cong WM,* et al. CSH guidelines for the diagnosis and treatment of drug-induced liver injury. Hepatol Int 2017; 11(3): 221–41. DOI: 10.1007/s12072-017-9793-2.
45. *Hey-Hadavi J, Seekins D, Palmer M, Coffey D, Caminis J, Abdullaev S,* et al. Overview of Causality Assessment for Drug-Induced Liver Injury (DILI) in Clinical Trials. Drug Saf 2021; 44(6): 619–34. DOI: 10.1007/s40264-021-01051-5.
46. *Moosa MS, Maartens G, Gunter H, Allie S, Chughlay MF, Setshedi M*, et al. Rechallenge after anti-tuberculosis drug-induced liver injury in a high HIV prevalence cohort. South Afr J HIV Med 2022; 23(1):1376. DOI: 10.4102/sajhivmed.v23i1.1376.
47. *Gezahegn LK, Argaw E, Assefa B, Geberesilassie A, Hagazi M*. Magnitude, outcome, and associated factors of anti-tuberculosis drug-induced hepatitis among tuberculosis patients in a tertiary hospital in North Ethiopia: A cross-sectional study. PLoS One 2020; 15(11): e0241346. DOI: 10.1371/journal.pone.0241346.
48. *Lim WS, Avery A, Kon OM, Dedicoat M*. Anti-tuberculosis drug-induced liver injury. BMJ 2023; 383: e074866. DOI: 10.1136/bmj-2023-074866. Erratum in: BMJ 2023; 383: p2806. DOI: 10.1136/bmj.p2806.
49. *National Institute for Health and Care Excellence (NICE)*. Tuberculosis: prevention, diagnosis, management and service organization [Internet]. London: NICE; 2016 [accessed 2026 Apr 30; cited 2026 May 14]. Available from: https://www.nice.org.uk/guidance/ng33
50. *Abbaspour F, Hasannezhad M, Khalili H, SeyedAlinaghi S, Jafari S*. Managing Hepatotoxicity Caused by Anti-tuberculosis Drugs: A Comparative Study of Approaches. Arch Iran Med 2024; 27(3): 122–6. DOI: 10.34172/aim.2024.19.
51. *Chiew AL, Gluud C, Brok J, Buckley NA*. Interventions for paracetamol (Acetaminophen) Overdose. Cochrane Database Syst Rev 2018; 2(2): CD003328. DOI: 10.1002/14651858.CD003328.pub3.
52. *Sanabria-Cabrera J, Tabbai S, Niu H, Alvarez-Alvarez I, Licata A, Björnsson E,* et al. N-Acetylcysteine for the Management of Non-Acetaminophen Drug-Induced Liver Injury in Adults: A Systematic Review. Front Pharmacol 2022; 13: 876868. DOI: 10.3389/fphar.2022.876868.
53. *Lewis JH, Korkmaz SY, Rizk CA, Copeland MJ*. Diagnosis, prevention and risk-management of drug-induced liver injury due to medications used to treat mycobacterium tuberculosis. Expert Opin Drug Saf 2024; 23(9): 1093–107. DOI: 10.1080/14740338.2024.2399074.
54. *Prasad S, Narang H, Kedia S, Ahuja V*. Anti-Tubercular Drug-Induced Liver Injury: Current Understanding and Emerging Directions. JGH Open 2026; 10(1): e70338. DOI: 10.1002/jgh3.70338.
55. *Azuma J, Ohno M, Kubota R, Yokota S, Nagai T, Tsuyuguchi K,* et al. NAT2 genotype guided regimen reduces isoniazid-induced liver injury and early treatment failure in the 6-month four-drug standard treatment of tuberculosis: a randomized controlled trial for pharmacogenetics-based therapy. Eur J Clin Pharmacol 2013; 69(5): 1091–101. DOI: 10.1007/s00228-012-1429-9.
56. *Sahiratmadja E, Rini IA, Penggoam S, Charles A, Maskoen AM, Parwati I*. Acetylator Status Among Newly Diagnosed and Recurrent Tuberculosis Patients from Kupang, Eastern Part of Indonesia. Pharmgenomics Pers Med 2021; 14: 737–44. DOI: 10.2147/PGPM.S311952.
57. *Akkahadsee P, Sawangjit R, Phumart P, Chaiyakunapruk N, Saklоetsakun D*. Systematic review and network meta-analysis of efficacy and safety of interventions for preventing anti-tuberculosis drug induced liver injury. Sci Rep 2023; 13(1): 19880. DOI: 10.1038/s41598-023-46565-3.

Received on March 29, 2026
Revised on April 20, 2026
Revised on May 9, 2026
Accepted on May 27, 2026
Online First July 2026

ORIGINAL ARTICLES

UDC: 616.24-002-07:616.24-008.4
DOI: https://doi.org/10.2298/VSP250704038L

Predictive value of the combination of lung ultrasound score, extravascular lung water index, and C-reactive protein/procalcitonin ratio for acute respiratory distress syndrome in patients with severe pneumonia

Prediktivna vrednost kombinacije skora ultrazvuka pluća, ekstravaskularnog indeksa vode u plućima i odnosa C-reaktivni protein/prokalcitonin za nastanak akutnog respiratornog distres sindroma kod obolelih od teške upale pluća

Zhu Lin*, Haisheng Liu†, Ning Luo*, Hua Xu*, Chenlin Zhu‡, Yanfei Zhu*, Hongfei Wang*

***Tianjin First Central Hospital, Department of Intensive Care Unit, Tianjin, China; †Yutian Traditional Chinese Medicine Hospital, Department of Intensive Care Unit, Tangshan, Hebei, China; ‡Nankai University, School of Medicine, Tianjin, China**

Abstract

Background/Aim. Discovering rapid and efficient biomarkers to predict the occurrence of acute respiratory distress syndrome (ARDS) is crucial for guiding subsequent treatment in patients with severe pneumonia (SP). The aim of this study was to determine the predictive value of the combination of the lung ultrasound score (LUS), the extravascular lung water index (EVLWI), and the C-reactive protein (CRP)/procalcitonin (PCT) ratio for predicting ARDS in patients with SP. **Methods.** The study included a total of 240 patients treated from January 2023 to January 2025, divided into two groups: a control group (n = 120) comprising patients with mild pneumonia and a research group (n = 120) comprising patients with SP. Comparisons of LUS, EVLWI, and CRP/PCT ratio were performed between the groups. The research group was further divided into an ARDS group (AG) and a non-ARDS group (N-AG). **Results.** Out of the 120 patients with SP, ARDS was registered in 20 (16.67%). The patients in AG exhibited significantly higher values of LUS, EVLWI, and CRP/PCT ratio in comparison to the N-AG patients ($p < 0.05$). In patients with SP, LUS, EVLWI, and CRP/PCT ratio positively correlated with the occurrence of ARDS ($r > 0$, $p < 0.05$) and were identified as independent risk factors for ARDS development (odds ratio > 1, $p < 0.05$). The areas under the receiver operating characteristic curve of LUS, EVLWI, CRP/PCT ratio, and their combination for predicting the occurrence of ARDS in patients with SP were 0.713, 0.739, 0.731, and 0.925 respectively (95% confidence interval: 0.867–0.983), with the combined model incorporating all three parameters showing the highest predictive performance. **Conclusion.** In patients with SP, LUS, EVLWI, and CRP/PCT ratio were elevated and were closely associated with the occurrence of ARDS.

Keywords:
biomarkers; c-reactive protein; pneumonia; prognosis; respiratory distress syndrome; ultrasonography.

Apstrakt

Uvod/Cilj. Otkrivanje brzih i efikasnih biomarkera za predviđanje pojave akutnog respiratornog distres sindroma (*acute respiratory distress syndrome* – ARDS) od ključnog je značaja za usmeravanje daljeg lečenja obolelih od teške upale pluća (*severe pneumonia* – SP). Cilj ovog rada bio je da se utvrdi prediktivna vrednost kombinacije skora ultrazvuka pluća (*lung ultrasound score* – LUS), ekstravaskularnog indeksa vode u plućima (*extravascular lung water index* – EVLWI) i odnosa C-reaktivni protein (CRP)/prokalcitonin (PCT) u prognozi ARDS kod obolelih od SP. **Metode.** Istraživanjem je obuhvaćeno ukupno 240 bolesnika, lečenih od januara 2023. do januara 2025. godine, koji su bili podeljeni u dve grupe: kontrolnu grupu (n = 120) u kojoj su bili oboleli od blage upale pluća i istraživačku grupu (n = 120) sa obolelima od SP. Izvršeno je poređenje vrednosti LUS, EVLWI i odnosa CRP/PCT između grupa. Istraživačka grupa je podeljena na ARDS grupu (AG) i grupu bez ARDS-a (N-AG). **Rezultati.** Od 120 obolelih od SP, ARDS je registrovan kod njih 20 (16,67%). Bolesnici iz AG imali su značajno povišene vrednosti LUS, EVLWI i odnos CRP/PCT u poređenju sa bolesnicima iz N-AG ($p < 0,05$). Kod obolelih od SP, vrednosti LUS, EVLWI i odnos CRP/PCT pozitivno su korelisali sa pojavom ARDS-a ($r > 0$, $p < 0,05$) i identifikovani su kao

Correspondence to: Hongfei Wang, Tianjin First Central Hospital, Department of Intensive Care Unit, Tianjin 300 192, China. E-mail: wanghftfch@chi-edu.cn

nezavisni faktori rizika od pojave ARDS-a (*odds ratio* > 1, $p < 0,05$). Površine ispod *receiver operating characteristic* krive za vrednosti LUS, EVLWI, odnos CRP/PCT i njihove kombinacije, za predviđanje pojave ARDS-a kod obolelih od SP, iznosile su 0,713, 0,739, 0,731 i 0,925 redom (95% *confidence interval*: 0,867–0,983), redom, pri čemu je kombinovani model koji je uključivao sva tri parametra pokazao najveću prediktivnu sposobnost. **Zaključak.** Kod obolelih od SP, vrednosti LUS, EVLWI i odnos CRP/PCT bili su povišeni i bili su usko povezani sa pojavom ARDS-a.

Ključne reči:
biomarkeri; c-reaktivni protein; pneumonija; prognoza; respiratorni distres sindrom; ultrasonografija.

Introduction

Severe pneumonia (SP), an infectious disease of the lungs caused by severe bacterial or viral infection, is characterized by severe clinical symptoms, rapid disease progression, and a high case fatality rate. Currently, a good prognosis is often achieved in most patients with SP following comprehensive therapies, including empirical anti-infective therapy, respiratory support (such as oxygen therapy and mechanical ventilation), and nutritional support. However, some patients still experience a poor prognosis even after comprehensive therapies and are prone to multiple severe complications [1, 2]. As a common and severe complication of SP, acute respiratory distress syndrome (ARDS) is characterized primarily by pathological alterations, including pulmonary edema (PE), alveolar capillary injury, pulmonary atelectasis, and hyaline membrane formation. Its clinical manifestations include progressive respiratory distress and refractory hypoxemia, seriously jeopardizing the life of patients [3, 4]. Clinically, therapeutic measures such as underlying disease treatment, respiratory and hemodynamic support, pulmonary protection strategies, immunomodulating therapy, and complication prevention are adopted for SP patients with ARDS, and these vary between patients with and without ARDS. Hence, discovering rapid and efficient biomarkers to predict the occurrence of ARDS in patients with SP is of great significance for guiding subsequent treatment strategies.

Previous studies have established that lung ultrasound score (LUS) reflects variations in pulmonary ventilation [5], while extravascular lung water (EVLW) index (EVLWI) quantifies PE and permeability impairment [6]. Additionally, inflammatory markers such as C-reactive protein (CRP) and procalcitonin (PCT), particularly the CRP/PCT ratio, effectively mirror the severity of systemic infection and tissue damage. While these parameters individually correlate with inflammatory lung diseases, their combined efficacy in predicting ARDS progression specifically in patients with SP remains to be elucidated.

The aim of this study was to determine the value of the combination of LUS, EVLWI, and CRP/PCT ratio for predicting the occurrence of ARDS in patients with SP.

Methods

Subjects

This study was approved by the Ethics Committee of Tianjin First Central Hospital, Tianjin, China No. (2021) GSFY [01], from January 27, 2023. The study included a total of 240 patients treated from January 2023 to January 2025. The patients were divided into two groups: a control group and a research group. The control group included 120 patients with mild pneumonia, while the research group included 120 SP patients receiving treatment therein in the same period. The mild pneumonia group was included as a reference group to compare baseline levels of LUS, EVLWI, and CRP/PCT ratio between different severities of pneumonia, whereas the primary analysis focused on ARDS prediction within the SP group.

Inclusion and exclusion criteria

The inclusion criteria for this study required that patients met the relevant diagnostic criteria of the Chinese Guidelines for the Diagnosis and Treatment of Adult Community-Acquired Pneumonia (2016 Edition) [7]. SP was defined by the presence of at least one major criterion–either the requirement for mechanical ventilation or septic shock requiring vasopressors–or at least three minor criteria, which included a respiratory rate ≥ 30 breaths/min, partial pressure of oxygen/fraction of inspired oxygen (PaO_2/FiO_2) ≤ 250 mmHg, multilobar infiltrates, or hypotension requiring intensive fluid resuscitation. Patients with confirmed pneumonia who did not meet these severe thresholds were assigned to the mild pneumonia group. Furthermore, eligible participants were required to have complete clinical and imaging data, a stable condition prior to enrollment, and no underlying history of severe immunosuppression. Finally, written informed consent was obtained from all patients or their legal representatives before participation.

The implemented exclusion criteria were as follows: patients with comorbid bronchial asthma, chronic obstructive pulmonary disease, lung tumors, tuberculosis, or other lung diseases; those with congenital airway dysplasia; those with congenital/genetic metabolic disorders; individuals who suffer from malnutrition; those with a history of glucocorticoid, antibiotic, or immunomodulator administration within the 3 months prior to enrollment; those with infectious or hematologic diseases.

Determination of lung ultrasound score

Ultrasonography was performed using a Versana Active scanner (GE HealthCare) equipped with a convex array probe. All post-processing features, including tissue harmonic imaging and spatial compound imaging, were deactivated during the examinations. LUS was measured by

a well-trained doctor from the intensive care unit. The bilateral anterior chest walls, lateral chest walls, and back were outlined along with the parasternal, anterior axillary, and posterior axillary lines, and the region was divided into upper and lower zones using the horizontal line between the nipples, resulting in 12 zones in total. Briefly, 0–3 points were allocated for each of the 12 pre-determined anatomical regions according to ultrasound pattern: normal = 0 points, well-defined B-lines = 1 point, coalescent B-lines = 2 points, consolidation = 3 points (total score: 0–36 points). Lung consolidations (3 points) were noted only when the thickness (perpendicular from the pleura) was greater than 15 mm. Sub-pleural thickening and sub-pleural consolidations (thickness 15 mm or thinner) were assigned a score of 2 points. Due to the observational nature of this study, strict blinding procedures were not formally implemented. However, LUS assessments and laboratory testing were performed independently in routine clinical practice, and clinicians performing LUS were blinded to the corresponding laboratory results during evaluation. The scores, along with details for each zone, were recorded in a table and kept in the patient's medical record to facilitate daily monitoring. LUS was evaluated daily when possible (depending on workload and available medical staff). For the purpose of analysis, the baseline LUS obtained at enrollment was used. The sum of the highest scores of the 12 zones based on ultrasonic findings was recorded as the total LUS.

Determination of extravascular lung water index

Following femoral artery puncture and catheterization, a pulse indicator continuous cardiac output system was utilized to monitor patient hemodynamics. The EVLWI value was measured by transpulmonary thermodilution three consecutive times, and the average value was recorded. For consistency with other variables, the EVLWI value obtained at enrollment (within 24 hrs of admission) was used for analysis.

Measurement of the C-reactive protein/procalcitonin ratio

Fasting venous blood (5 mL) was harvested on the day of enrollment, left at room temperature for 30 min, and centrifuged to isolate the serum. Next, serum concentrations of CRP (Biopike, USCN-HEA821Bo, reference range: 0–5 mg/L) and PCT (EK-Bioscience, Shanghai, China, AB6036, reference range: 0–0.5 μg/L) were determined *via* a double-antibody sandwich enzyme-linked immunosorbent assay (ELISA). The CRP/PCT ratio was calculated by dividing the CRP value (mg/L) by the PCT value (μg/L), without additional unit conversion.

Determination of the occurrence of acute respiratory distress syndrome

ARDS was diagnosed according to the 2012 Berlin definition, which includes the following: acute onset within 1 week of a known clinical insult or new/worsening respiratory symptoms; bilateral pulmonary opacities on chest X-ray imaging that cannot be fully explained by pleural effusions, lobar or lung collapse, or pulmonary nodules; impaired oxygenation classified as mild (200 mmHg < PaO_2/FiO_2 ≤ 300 mmHg), moderate (100 mmHg < PaO_2/FiO_2 ≤ 200 mmHg), and severe (PaO_2/FiO_2 ≤ 100 mmHg). The diagnosis of ARDS was independently established by attending intensive care physicians. Given the observational nature of this study, the diagnosing clinicians had access to patients' routine medical records and were not strictly blinded to the baseline LUS, EVLWI, and CRP/PCT results. However, all diagnoses were strictly adjudicated according to the objective criteria of the 2012 Berlin definition [8].

Collection of general data

The gender distribution, age, body mass index (BMI), respiratory rate, heart rate, blood pressure, oxygen saturation, and arterial lactate values in the ARDS group (AG) and non-ARDS group (N-AG) were recorded. In addition, all predictor variables (LUS, EVLWI, and CRP/PCT) were measured at enrollment (within 24 hrs of admission), prior to the development of ARDS, and were used in subsequent predictive analyses. The occurrence of ARDS was prospectively monitored during hospitalization.

Statistical analysis

For this analysis, SPSS 23.0 software was used. Measurement data were expressed as mean ± standard deviation and examined using the *t*-test. Continuous variables are expressed as median (interquartile range – IQR). Logistic regression (LR) analysis was conducted to identify risk factors for the occurrence of ARDS in patients with SP. The correlations between LUS, EVLWI, and CRP/PCT ratio and the occurrence of ARDS in patients with SP were evaluated using Spearman correlation analysis. Receiver operating characteristic (ROC) curves were constructed to evaluate the predictive performance of LUS, EVLWI, CRP/PCT ratio, and their combination for the occurrence of ARDS in patients with SP. Areas under the curve (AUC) > 0.90, 0.71–0.90, 0.50–0.70, and < 0.50 were considered indicative of high, fair, low, and no predictive accuracy, respectively. A p-value < 0.05 was considered statistically significant. Variables with $p < 0.05$ in univariate analysis were included in the multivariable LR model. Model calibration was evaluated using the Hosmer-Lemeshow goodness-of-fit test, with a $p > 0.05$ indicating acceptable calibration. A sensitivity analysis was conducted by forcing clinically important covariates (including age, oxygen saturation, and arterial lactate value) to assess the robustness of the primary predictors.

Results

A control group comprised 120 patients with mild pneumonia, including 68 males and 52 females aged 40–75

years (mean 50.32 ± 5.28 years), with a BMI of 20–26 kg/m^2 (mean 24.86 ± 0.55 kg/m^2). Besides, a research group was set up to incorporate 120 SP patients receiving treatment therein in the same period, including 70 males and 50 females aged 38–76 years old, with a mean of 50.36 ± 5.46 years old and a BMI of 20–26 kg/m^2 (average 25.03 ± 0.52 kg/m^2). The gender distribution, age, and BMI were comparable between the two groups ($p > 0.05$).

LUS, EVLWI, and CRP/PCT ratio were higher in the research group than in the control group ($p < 0.05$) (Table 1).

Among the 120 patients with SP, 20 (16.67%) had ARDS. Among them, the median interval from baseline predictor measurement to ARDS diagnosis was 2.3 days (IQR: 1–4 days).

The gender distribution, age, BMI, respiratory rate, heart rate, mean arterial pressure, oxygen saturation, and arterial lactate level showed no statistically significant differences between AG and N-AG ($p > 0.05$), whereas LUS, EVLWI, and CRP/PCT ratio were increased in AG compared to those in N-AG. LUS, EVLWI, and CRP/PCT ratio may provide additional predictive value ($p < 0.05$) (Table 2).

The results of Spearman's rank correlation analysis revealed that LUS, EVLWI, and CRP/PCT ratio were positively correlated with the occurrence of ARDS in patients with SP ($\rho > 0$, $p < 0.05$) (Table 3).

Table 1

LUS, EVLWI, and CRP/PCT ratio in research and control groups

Group	LUS, point	EVLWI, mL/kg	CRP/PCT ratio
Research (n = 120)	15.96 ± 2.10	13.89 ± 1.23	9.38 ± 1.03
Control (n = 120)	8.80 ± 1.15	10.75 ± 0.90	6.35 ± 0.52
p-value	< 0.001	< 0.001	< 0.001

LUS – lung ultrasound score; EVLWI – extravascular lung water index; CRP – C-reactive protein; PCT – procalcitonin; n – number of respondents.
All values are given as mean ± standard deviation.

Table 2

Relevant indicators in ARDS and non-ARDS groups

Indicator	N-AG (n = 100)	AG (n = 20)	*p*-value*
Gender			
male	59 (59.00)	11 (55.00)	0.741
female	41 (41.00)	9 (45.00)	
Age, year	50.32 ± 3.28	51.30 ± 3.25	0.224
Body mass index, kg/m^2	24.86 ± 0.54	24.81 ± 0.52	0.705
Respiratory rate, breaths/min	37.52 ± 5.28	35.56 ± 5.27	0.132
Heart rate, beats/min	100.00 ± 15.52	98.03 ± 14.53	0.602
Mean arterial pressure, mmHg	90.50 ± 5.03	90.51 ± 5.04	0.994
Oxygen saturation, %	93.60 ± 7.04	92.59 ± 7.15	0.560
Arterial lactate value, mmol/L	2.45 ± 0.25	2.50 ± 0.26	0.419
LUS, point	15.02 ± 2.04	20.66 ± 3.20	< 0.001
EVLWI, mL/kg	13.21 ± 1.10	17.29 ± 2.08	< 0.001
CRP/PCT ratio	8.50 ± 0.96	13.78 ± 1.05	< 0.001

ARDS – acute respiratory distress syndrome; N-AG – non-ARDS group; AG – ARDS group; n–number of respondents; LUS – lung ultrasound score; EVLWI – extravascular lung water index; CRP – C-reactive protein; PCT – procalcitonin.
All values are given as numbers (percentages) or mean ± standard deviation.
***Note*: *Continuous variables were compared using the *t*-test, whereas categorical variables were compared using the chi-square test.**

Table 3

Correlations analysis of LUS, EVLWI, and CRP/PCT ratio with the occurrence of ARDS in patients with severe pneumonia

Coefficient	LUS	EVLWI	CRP/PCT
ρ	0.561	0.603	0.646
p-value	< 0.001	< 0.001	< 0.001

LUS – lung ultrasound score; EVLWI – extravascular lung water index; CRP – C-reactive protein; PCT – procalcitonin; ARDS – acute respiratory distress syndrome.

Table 4

Results of logistic regression analysis on the occurrence of ARDS in severe pneumonia patients

Item	B	SE	Wald	*p*-value	OR	95% CI
LUS	1.005	0.226	19.845	< 0.001	2.733	1.756–4.253
EVLWI	2.130	0.541	15.521	< 0.001	8.416	2.917–24.285
CRP/PCT	0.577	0.121	22.559	< 0.001	1.780	1.403–2.258

ARDS – acute respiratory distress syndrome; SE – standard error; OR – odds ratio; CI – confidence interval; LUS – lung ultrasound score; EVLWI – extravascular lung water index; CRP – C-reactive protein; PCT – procalcitonin.

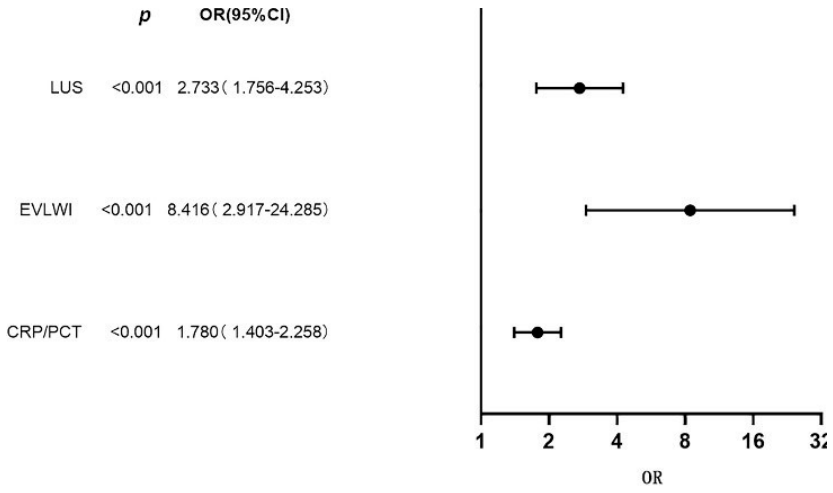

Fig. 1 – Forest plot of clinical characteristics based on multivariate logistic regression analysis. OR – odds ratio; CI – confidence interval; LUS – lung ultrasound score; EVLWI – extravascular lung water index; CRP – C-reactive protein; PCT – procalcitonin.

Table 5

Value of LUS, EVLWI, CRP/PCT ratio, and their combination for predicting the occurrence of ARDS in patients with severe pneumonia

Item	Optimal cut-off value	AUC	SE	*p*-value	95% CI	Sensitivity	Specificity	Youden index
LUS	16.955 points	0.713	0.076	0.003	0.563–0.862	0.700	0.730	0.430
EVLWI	14.690 mL/kg	0.739	0.073	0.001	0.596–0.883	0.800	0.770	0.570
CRP/PCT	10.635 ratio	0.731	0.072	0.001	0.590–0.872	0.850	0.740	0.590
Combination	-	0.925	0.030	< 0.001	0.867–0.983	0.900	0.840	0.740

LUS – lung ultrasound score; EVLWI – extravascular lung water index; CRP – C-reactive protein; PCT – procalcitonin; ARDS – acute respiratory distress syndrome; AUC – area under the curve; SE – standard error; CI – confidence interval.

According to LR analysis, LUS, EVLWI, and CRP/PCT ratio served as risk factors for the occurrence of ARDS in patients with SP [odds ratio (OR) > 1, $p < 0.05$].

Twenty ARDS events and three predictors were included in the primary LR model, resulting in an events-*per*-variable ratio of approximately 6.7.

EVLWI showed the strongest association [OR = 8.416, 95% confidence interval (CI): 2.917–24.285], followed by LUS (OR = 2.733, 95% CI: 1.756–4.253) and CRP/PCT ratio (OR = 1.780, 95% CI: 1.403–2.258) (Table 4 and Figure 1). Furthermore, an exploratory sensitivity analysis was performed by additionally adjusting for clinically important covariates, including age, oxygen saturation, and arterial lactate value. In this adjusted model, LUS, EVLWI, and the CRP/PCT ratio remained independently associated with the occurrence of ARDS ($p < 0.05$), and the direction of the associations was consistent with that observed in the primary model, supporting the robustness of these predictors after adjustment for selected baseline clinical covariates.

The ROC curves were plotted using the presence/absence of ARDS in patients with SP (0 = absence, and 1 = presence) as the state variable, together with LUS, EVLWI, and CRP/PCT ratio as the test variables. The results uncovered that the AUCs of LUS, EVLWI, CRP/PCT ratio, and their combination were 0.713 (95% CI: 0.563–0.862), 0.739 (95% CI: 0.596–0.883), 0.731 (95% CI: 0.590–0.872), and 0.925 (95% CI: 0.867–0.983), respectively (Table 5 and Figure 2). The Hosmer-Lemeshow goodness-of-fit test showed no significant evidence of poor calibration for the combined prediction model, with no significant difference between the observed and predicted probabilities ($\chi^2 = 5.684$, $df = 8$, $p = 0.683$).

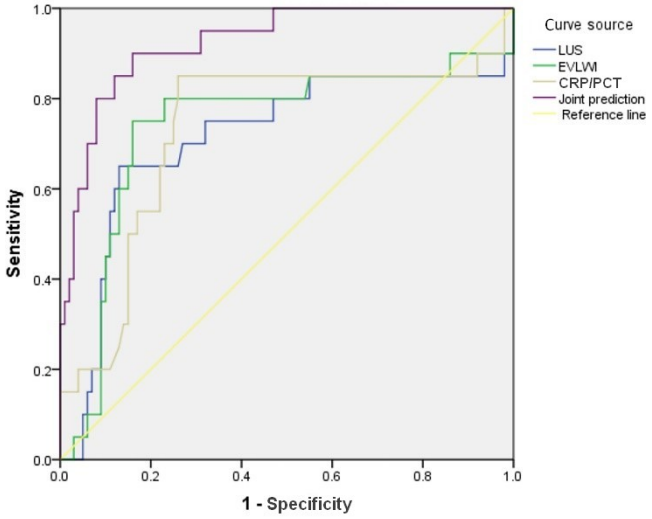

Fig. 2 – ROC curves of LUS, EVLWI, CRP/PCT ratio, and their combination for predicting the occurrence of ARDS in patients with severe pneumonia. ROC – receiver operating characteristic curves; LUS – lung ultrasound score; EVLWI – extravascular lung water index; CRP – C-reactive protein; PCT – procalcitonin; ARDS – acute respiratory distress syndrome.

Discussion

As a serious lung infection, SP covers extensive inflammation and damage to the lungs, which can result in severe complications and enhance the medical and economic burdens. As a common complication of SP in the clinic, ARDS can significantly worsen the illness, enhance the difficulties in infection control and clinical treatment, and increase the mortality rate [9]. Therefore, discovering biomarkers for efficient prediction of ARDS in patients with SP is of great significance for guiding the selection of clinical therapeutic regimens.

LUS, EVLWI, and CRP/PCT ratio were increased in AG, suggesting that conventional clinical indicators alone may be insufficient to distinguish ARDS risk. However, the combination of LUS, EVLWI, and CRP/PCT ratio may provide additional predictive value ($p < 0.05$).

EVLWI is an important parameter for assessing PE and pulmonary permeability impairment, and provides useful information for lung status evaluation and fluid management. A recent systematic review and meta-analysis showed that higher EVLW measured by transpulmonary thermodilution was associated with increased mortality in critically ill patients, supporting the prognostic value of EVLWI in acute lung injury and ARDS-related conditions [10]. EVLWI has also been reported to predict prognosis in patients with acute lung injury in China [11]. In the present study, EVLWI was higher in AG than in N-AG and was positively associated with the occurrence of ARDS. LR analysis further showed that EVLWI was an independent risk factor for ARDS in patients with SP. This may be explained by the disruption of the alveolar-capillary barrier in SP, which increases vascular permeability and promotes PE formation [12–14]. As a quantitative indicator of EVLW, EVLWI reflects the degree of lung water accumulation and pulmonary permeability impairment [15, 16], making its association with ARDS biologically plausible.

LUS is a noninvasive tool for evaluating changes in pulmonary aeration by quantifying lung ultrasound findings. A previous study has shown that LUS changes with the progression of pneumonia and the extent of pulmonary involvement, and may be useful for prognostic assessment [17]. In this study, LUS was significantly higher in AG than in N-AG and was positively associated with the occurrence of ARDS. LR analysis also identified increased LUS as a risk factor for ARDS. These findings are consistent with previous evidence and may be explained by the ability of LUS to reflect diffuse alveolar damage, loss of aeration, and fluid accumulation in the lungs [18–20]. Therefore, a higher LUS may indicate more severe pulmonary involvement and a greater likelihood of progression to ARDS.

CRP and PCT are commonly used inflammatory biomarkers. CRP is an acute-phase protein synthesized by the liver in response to inflammatory stimuli, whereas PCT increases mainly in bacterial infection and systemic inflammatory responses. In the present study, the CRP/PCT ratio was higher in AG than in N-AG and was identified as a risk factor for ARDS. This may be because CRP and PCT reflect different aspects of inflammatory and infectious responses, and an elevated CRP/PCT ratio may indicate aggravated inflammation, tissue injury, and infection burden in SP [21, 22].

With disease progression, this inflammatory imbalance may further contribute to the development of ARDS [23, 24].

In the present study, the AUCs of LUS, EVLWI, CRP/PCT ratio, and their combination for predicting ARDS in patients with SP were 0.713, 0.739, 0.731, and 0.925, respectively, indicating that the combined model exhibited superior predictive performance compared with any individual indicator. This result appears favorable compared

with previously published models. For example, a multicenter lung ultrasound-based ARDS model reported an AUC of 0.90 in the derivation cohort and 0.80 in the validation cohort [5]. In contrast, a nomogram for predicting ARDS in patients with bacterial pneumonia achieved an AUC of 0.794 [25]. A sepsis-associated ARDS prediction model reported AUCs of 0.811 and 0.812 in the training and testing sets, respectively [26]. Although direct comparisons across studies should be interpreted with caution due to differences in study populations, predictor composition, and validation strategies, the combined model in the present study showed promising predictive performance. Clinically, this combination may help identify patients with SP who are at high risk of ARDS and may benefit from closer respiratory and hemodynamic monitoring, earlier optimization of fluid management, and timely escalation of supportive care.

Limitation of the study

This study has several limitations. First, although LUS and EVLWI can be dynamically monitored during clinical management, only baseline measurements obtained at enrollment were included in the present analysis. Therefore, the potential effects of treatment interventions, such as fluid management, ventilatory support, and anti-infective therapy, were not evaluated. Second, although several baseline clinical variables were collected, residual confounding could not be completely excluded. Third, the relatively small number of ARDS events may have affected model stability. In this study, 20 ARDS events and 3 predictors were included in the primary LR model, resulting in an events-*per*-variable ratio of approximately 6.7, which is fewer than the traditionally recommended threshold of 10. Therefore, the model may be at risk of overfitting and should be interpreted as exploratory. In addition, internal validation using bootstrapping was not performed, and an independent external validation cohort was not available. Finally, strict blinding was not formally implemented in this real-world clinical setting, which may have introduced potential observational bias.

Conclusion

The present findings suggest that elevated lung ultrasound score, extravascular lung water index, and C-reactive protein/procalcitonin ratio are associated with the occurrence of acute respiratory distress syndrome in patients with severe pneumonia. Their combined use may enhance predictive accuracy for the occurrence of acute respiratory distress syndrome in these patients. Future studies with larger cohorts, bootstrapping-based internal validation, and multicenter external validation are warranted to confirm the robustness and generalizability of the model.

Conflicts of interest

The authors declare no conflict of interest.

Funding

This study was financially supported by the Tianjin Medical Construction of Key Discipline (Specialty) Funded Project (No. TJYXZDXK-013A) and the Tianjin Science and Technology Program (No. 21JCYBJC00090).

REFERENCES

1. *Martin-Loeches I, Torres A, Nagavci B, Aliberti S, Antonelli M, Bassetti M,* et al. ERS/ESICM/ESCMID/ALAT guidelines for the management of severe community-acquired pneumonia. Intensive Care Med 2023; 49(6): 615–32. DOI: 10.1007/s00134-023-07033-8. Erratum in: Intensive Care Med 2023; 49(8): 1040–1. DOI: 10.1007/s00134-023-07082-z.
2. *Vaughn VM, Dickson RP, Horowitz JK, Flanders SA.* Community-Acquired Pneumonia: A Review. JAMA 2024; 332(15): 1282–95. DOI: 10.1001/jama.2024.14796.
3. *Matthay MA, Arabi Y, Arroliga AC, Bernard G, Bersten AD, Brochard LJ,* et al. A New Global Definition of Acute Respiratory Distress Syndrome. Am J Respir Crit Care Med 2024; 209(1): 37–47. DOI: 10.1164/rccm.202303-0558WS.
4. *Yang P, Sjoding MW.* Acute Respiratory Distress Syndrome: Definition, Diagnosis, and Routine Management. Crit Care Clin 2024; 40(2): 309–27. DOI: 10.1016/j.ccc.2023.12.003.
5. *Smit MR, Hagens LA, Heijnen NFL, Pisani L, Cherpanath TGV, Dongelmans DA,* et al. Lung Ultrasound Prediction Model for Acute Respiratory Distress Syndrome: A Multicenter Prospective Observational Study. Am J Respir Crit Care Med 2023; 207(12): 1591–601. DOI: 10.1164/rccm.202210-1882OC.
6. *Meng W, Zhang C, Hu J, Tang Z.* Evaluation of extravascular lung water index in critically ill patients based on lung ultrasound radiomics analysis combined with machine learning. Zhonghua Wei Zhong Bing Ji Jiu Yi Xue 2023; 35(10): 1074–9. DOI: 10.3760/cma.j.cn121430-20230209-00077. (Chinese)
7. *Cao B, Huang Y, She DY, Cheng QJ, Fan H, Tian XL,* et al. Diagnosis and treatment of community-acquired pneumonia in adults: 2016 clinical practice guidelines by the Chinese Thoracic Society, Chinese Medical Association. Clin Respir J. 2018 12(4): 1320-1360. DOI:10.1111/crj.12674.
8. *ARDS Definition Task Force; Ranieri VM, Rubenfeld GD, Thompson BT, Ferguson ND, Caldwell E,* et al. Acute respiratory distress syndrome: the Berlin Definition. JAMA 2012; 307(23): 2526–33. DOI: 10.1001/jama.2012.5669.
9. *Cave C, Samano D, Sharma AM, Dickinson J, Salomon J, Mahapatra S.* Acute respiratory distress syndrome: A review of ARDS across the life course. J Investig Med 2024; 72(8): 798–818. DOI: 10.1177/10815589241270612.
10. *Gavelli F, Shi R, Teboul JL, Azzolina D, Mercado P, Jozwiak M,* et al. Extravascular lung water levels are associated with mortality: a systematic review and meta-analysis. Crit Care 2022; 26(1): 202. DOI: 10.1186/s13054-022-04061-6.
11. *Jiang Z, Wu J, Wan M, Liu L, Zhou X.* Evaluation of the prognostic value of lncRNA UCA1 combined with extravascular lung water index and lung ultrasound score in patients with acute lung injury. Clin Respir J 2023; 17(7): 654–62. DOI: 10.1111/crj.13651.
12. *Meegan JE, Rizzo AN, Schmidt EP, Bastarache JA.* Cellular Mechanisms of Lung Injury: Current Perspectives. Clin Chest Med 2024; 45(4): 821–33. DOI: 10.1016/j.ccm.2024.08.004.

13. *Xu H, Sheng S, Luo W, Xu X, Zhang Z.* Acute respiratory distress syndrome heterogeneity and the septic ARDS subgroup. Front Immunol 2023; 14: 1277161. DOI: 10.3389/fimmu.2023.1277161.
14. *Zhang PH, Zhang WW, Wang SS, Wu CH, Ding YD, Wu XY,* et al. Efficient pulmonary lymphatic drainage is necessary for inflammation resolution in ARDS. JCI Insight 2024; 9(1): e173440. DOI: 10.1172/jci.insight.173440.
15. *You JY, Guo W, Geng L, Huang J, Shen YL, Zhang Q.* Beneficial effects of extravascular lung water index-guided volume management in patients with cardiogenic shock. Zhonghua Xin Xue Guan Bing Za Zhi 2024; 52(9): 1081–9. DOI: 10.3760/cma.j.cn112148-20240213-00093. (Chinese)
16. *Gonzalez-Plascencia M, Martinez-Fierro ML, Salazar de Santiago A, Castañeda-Miranda AG, Badillo-Almaraz JI, Garza-Veloz I.* Current Insights into Clinical, Molecular, and Therapeutic Approaches to Acute Respiratory Distress Syndrome. Med Sci (Basel) 2026; 14(1): 134. DOI: 10.3390/medsci14010134.
17. *Fu B, Zhang P, Zhang J.* Diagnosis and Prognosis Evaluation of Severe Pneumonia by Lung Ultrasound Score Combined with Serum Inflammatory Markers. Mediterr J Hematol Infect Dis 2023; 15(1): e2023057. DOI: 10.4084/MJHID.2023.057.
18. *Maddaloni C, De Rose DU, Ronci S, Bersani I, Martini L, Caoci S,* et al. Lung Ultrasound Score in Neonates with Congenital Diaphragmatic Hernia (CDH-LUS): A Cross-Sectional Study. Diagnostics (Basel) 2023; 13(5): 898. DOI: 10.3390/diagnostics13050898.
19. *Chidini G, Raimondi F.* Lung ultrasound for the sick child: less harm and more information than a radiograph. Eur J Pediatr 2024; 183(3): 1079–89. DOI: 10.1007/s00431-023-05377-3.
20. *De Luca D, Bonadies L, Alonso-Ojembarrena A, Martino D, Gutierrez-Rosa I, Loi B,* et al. Quantitative Lung Ultrasonography to Guide Surfactant Therapy in Neonates Born Late Preterm and Later. JAMA Netw Open 2024; 7(5): e2413446. DOI: 10.1001/jamanetworkopen.2024.13446. Erratum in: JAMA Netw Open 2024; 7(10): e2446284. DOI: 10.1001/jamanetworkopen.2024.46284.
21. *Zhou J, Jin F, Wu F.* Clinical significance of changes in serum inflammatory factors in patients with chronic obstructive pulmonary disease and pulmonary infection. J Int Med Res 2021; 49(5): 3000605211013275. DOI: 10.1177/03000605211013275.
22. *VanDevanter DR, Heltshe SL, Skalland M, West NE, Sanders DB, Goss CH,* et al. C-reactive protein (CRP) as a biomarker of pulmonary exacerbation presentation and treatment response. J Cyst Fibros 2022; 21(4): 588–93. DOI: 10.1016/j.jcf.2021.12.003.
23. *Li Y, Min LF, Zhang X.* Usefulness of procalcitonin (PCT), C-reactive protein (CRP), and white blood cell (WBC) levels in the differential diagnosis of acute bacterial, viral, and mycoplasmal respiratory tract infections in children. BMC Pulm Med 2021; 21(1): 386. DOI: 10.1186/s12890-021-01756-4.
24. *Schuetz P.* How to best use procalcitonin to diagnose infections and manage antibiotic treatment. Clin Chem Lab Med 2022; 61(5): 822–8. DOI: 10.1515/cclm-2022-1072.
25. *Lv L, Shao X, Cui E.* Establishment of a Predictive Model for Acute Respiratory Distress Syndrome in Patients with Bacterial Pneumonia. J Inflamm Res 2024; 17: 2825–34. DOI: 10.2147/JIR.S458690.
26. *Xu C, Zheng L, Jiang Y, Jin L.* A prediction model for predicting the risk of acute respiratory distress syndrome in sepsis patients: a retrospective cohort study. BMC Pulm Med 2023; 23(1): 78. DOI: 10.1186/s12890-023-02365-z.

Received on July 4, 2025
Revised on March 6, 2026
Revised on May 7, 2026
Revised on May 19, 2026
Accepted on May 29, 2026
Online First July 2026

ORIGINAL ARTICLE

UDC: 613.84(4)"2014/2019"
DOI: https://doi.org/10.2298/VSP251210036D

Stagnation and inequality: a pan-European analysis of structural determinants in smoking prevalence (2014–2019)

Stagnacija i nejednakost: panevropska analiza strukturnih činilaca u prevalenciji pušenja (2014–2019)

Ibrahim Demir*, Ozkan Ayvaz†

***Istanbul Provincial Health Directorate, Fatih, Istanbul, Türkiye; †Istanbul University, Istanbul Faculty of Medicine, Department of Public Health, Fatih, Istanbul, Türkiye**

Abstract

Background/Aim. Although the global prevalence of smoking has been steadily declining, aggregate trends conceal substantial regional differences across Europe, as well as socioeconomic and education-level inequalities**.** The aim of this study was to assess the widening regional and educational inequalities in smoking prevalence in Europe and delineate structural barriers to tobacco control against the backdrop of aggregate trends. **Methods.** Using European Statistical Office (Eurostat) data from 32 countries (2014–2019), daily smoking rates, indoor environmental tobacco smoke exposure, and smoking intensity were analyzed. To account for demographic differences, European averages were calculated using population-weighted means. Pearson correlations and multivariate regression models were utilized to examine country-level associations between educational attainment indices and smoking outcomes. **Results.** While the weighted European Union (EU) average for daily smoking declined slightly, regional divergence intensified. Nordic countries maintained the lowest prevalence (~12%), whereas Eastern European nations and Türkiye (~27–29%) exhibited stagnation or increases. A strong country-level educational gradient persisted; nations with higher tertiary attainment rates exhibited significantly lower aggregate smoking prevalence. Marked cross-country differences in indoor secondhand smoke exposure were observed; daily indoor tobacco smoke exposure exceeded 19% in countries such as Greece and Croatia but remained below 3% in Finland. **Conclusion.** The decline in smoking has stalled in structurally disadvantaged regions, supporting the "hardening hypothesis" (the phenomenon where the remaining smoker population becomes more resistant to quitting) at the macro level. Generic tobacco control measures are losing efficacy. To bridge the widening health equity gap, future interventions must target high levels of heavy smoking and the enforcement of smoke-free environments in lagging regions.

Keywords:
education; europe; prevalence; smoke-free policy; tobacco smoke pollution; tobacco smoking.

Apstrakt

Uvod/Cilj. Iako se prevalencija pušenja na globalnom nivou postepeno smanjuje, ukupni trendovi maskiraju značajne nejednakosti između regiona u Evropi, kao i nejednakosti u zavisnosti od obrazovnog i socioekonomskog statusa. Cilj ove studije bio je da proceni da li se nejednakosti u prevalenciji pušenja u Evropi, koje zavise od regionalne pripadnosti i obrazovnog statusa, povećavaju i da identifikuje strukturne barijere za kontrolu upotrebe duvana u kontekstu ukupnih trendova. **Metode.** Korišćenjem podataka Evropskog zavoda za statistiku (*European Statistical Office* – Eurostat) iz 32 zemlje (2014–2019), analizirane su dnevne stope pušenja, izloženost duvanskom dimu u zatvorenom prostoru i intenzitet pušenja. Kako bi se uzele u obzir demografske razlike, evropski proseci su izračunati kao srednje vrednosti ponderisane prema broju stanovnika. Za ispitivanje povezanosti između indeksa nivoa obrazovanja i pokazatelja u vezi sa pušenjem na nivou zemlje korišćeni su Pirsonove korelacije i multivarijantni regresioni modeli. **Rezultati.** Iako je ponderisani prosek prevalencije svakodnevnog pušenja u Evropskoj uniji (EU) blago opao, regionalne razlike su se pojačale. Nordijske zemlje zadržale su najnižu prevalenciju (~12%), dok su u istočnoevropskim zemljama i Turskoj primećeni stagnacija ili porast (~27–29%). Velika obrazovna razlika na nivou zemalja je postojala; zemlje sa većim udelom visokoobrazovanog stanovništva imale su značajno nižu ukupnu prevalenciju pušenja. Uočene su izražene razlike među evropskim zemljama u izloženosti duvanskom dimu u zatvorenom prostoru; u Grčkoj i Hrvatskoj svakodnevna izloženost prelazila je 19%, dok je u Finskoj iznosila manje od 3%. **Zaključak.** Pad stope pušenja stagnirao je u strukturno nepovoljnim regionima, što podržava „hipotezu o očvršćavanju" (fenomen u kojem preostala

Correspondence to: Ibrahim Demir, Istanbul Provincial Health Directorate, Binbirdirek, Peykhane St. No. 8, Fatih, 34 122, Istanbul, Türkiye. E-mail: ibrahimdem72@gmail.com

populacija pušača postaje otpornija na prestanak pušenja) na makro nivou. Generičke mere kontrole duvana gube efikasnost. Da bi se premostio sve veći jaz u zdravstvenoj jednakosti, buduće intervencije moraju biti usmerene prema visokom nivou „teškog“ pušenja i sprovođenju okruženja bez duvanskog dima u regionima koji zaostaju.

Ključne reči:
obrazovanje; evropa; prevalencija; pušenje, politika zabrane; zagađenje duvanskim dimom; pušenje.

Introduction

Tobacco smoking persists as a paramount global public health challenge, implicated in over 8 million deaths annually and imposing a massive burden of preventable disease and disability [1]. Since its adoption, the World Health Organization's (WHO) Framework Convention on Tobacco Control has provided a blueprint for evidence-based policy interventions, contributing to a gradual decline in global smoking prevalence (SP) [2]. However, this aggregate progress conceals a landscape marked by deep and persistent inequalities. The declining prevalence is not uniformly distributed across populations; instead, it is increasingly concentrated within specific socioeconomic and geographic groups, thereby exacerbating health disparities [3, 4]. The inverse social gradient of smoking is one of the most robust findings in social epidemiology [5]. Socioeconomic status (SES), frequently operationalized through educational attainment, functions as a "fundamental cause" of health outcomes, shaping access to resources, knowledge, and social networks that either protect against or promote health-risk behaviors [6]. Individuals with lower education are more likely to initiate smoking, less likely to quit, and suffer a disproportionately higher burden of tobacco-related morbidity and mortality [7, 8]. This gradient is not merely a reflection of individual choice but is embedded in a context of differential exposure to stress, targeted industry marketing, and varying social norms [9, 10]. Beyond individual socioeconomic positioning, the broader environmental and policy context plays a critical role. Exposure to secondhand smoke (SHS) in indoor environments is not only a direct cause of disease in non-smokers but also serves as a powerful cue that normalizes smoking behavior, potentially reinforcing social norms and undermining cessation efforts [11, 12]. The implementation and enforcement of comprehensive smoke-free legislation have been shown to reduce both exposure and consumption, yet the strength of these laws varies dramatically across and within regions, creating distinct geographical patterns of SP [13, 14]. Comparative studies within Europe consistently reveal a stark divide, with Nordic nations demonstrating sustained low prevalence–a success attributed to decades of comprehensive, multifaceted tobacco control–while countries in Eastern Europe (EE) often exhibit persistently high rates, linked to historical legacies and slower policy adoption [15, 16]. While the individual associations between education, policy, environment, and smoking are well-documented in siloed studies, there remains a critical need for integrated analyses that examine these multilevel factors concurrently. Such an approach allows for the quantification of their unique and combined contributions to SP, providing a more holistic understanding of the drivers of both overall trends and embedded inequalities [17, 18].

This study aims to address a critical research gap by conducting a comprehensive, cross-national analysis with four specific objectives: first, to delineate the descriptive epidemiology of smoking status and its correlation with education; second, to analyze temporal trends in the prevalence of daily smoking (DS) and non-smoking between 2014 and 2019; third, to investigate and quantify regional variations in DS rates across European sub-regions; and finally, to construct a multivariate model that integrates educational, environmental, and temporal factors to identify the key predictors of DS prevalence.

Methods

Ethical approval for this research was not required as this study was conducted using publicly available data.

Study design and data source

This study employed a longitudinal ecological design utilizing country-level aggregated data (dataset category: TOTAL) obtained from the Eurostat database [19]. The initial sample encompassed 32 European countries/regions. The analysis focused on two time points (2014 and 2019) and included countries based on data availability for the variables of interest. Given the ecological nature of the study, all analyses were conducted at the country level; consequently, the findings cannot be interpreted at an individual level.

Sample construction and management of missing data

Different analytic procedures require different sets of variables; therefore, sample sizes vary across analyses. To ensure internal validity, a complete-case (listwise deletion) approach was used for each analysis, excluding countries with missing data on the variables used in each statistical test. The descriptive analysis (Table 1) included all available country-year observations with non-missing data, yielding up to 64 observations from 32 countries across both time points. For the paired samples *t*-tests (2014 vs. 2019), the analysis was based on a balanced panel of 28 countries with complete data for both time points (n = 56 country-year observations). Countries with missing data in either year–such as the United Kingdom (UK) for 2019 and Serbia for 2014–were excluded from this specific analysis. The regression analysis encompassed 32 countries (n = 62 country-year observations). This sample reflects available data for all predictors, allowing for the partial inclusion of countries

Table 1

Descriptive statistics of smoking behavior, exposure, and educational attainment in the sample

Variable (n)	Mean ± SD	Min–Max
Cigarette smokers (64)		
daily	19.58 ± 5.92	6.40–29.20
occasional	5.12 ± 1.95	1.70–8.30
non-smokers	75.30 ± 5.41	63.70–88.70
Former smokers (32)	27.31 ± 11.21	10.70–66.30
Indoor tobacco smoke exposure daily (48)	10.89 ± 7.45	1.50–37.90
Cigarette consumption		
E-cigarettes daily (32)	1.59 ± 1.12	0.30–4.60
< 20 cigarettes/day (64)	12.79 ± 3.15	5.30–19.60
≥ 20 cigarettes/day (64)	6.54 ± 3.92	0.00–15.80
Education level (32)		
low	19.28 ± 5.12	11.30–31.80
medium	21.89 ± 6.78	14.20–34.10
high	13.45 ± 6.23	3.60–23.80

n – number of observations; SD – standard deviation; Min – minimum; Max – maximum; E-cigarette – electronic cigarette.
All values are represented in percentages.

where only one time point was available, such as the UK in 2014 and Serbia in 2019. Furthermore, the SHS exposure sub-sample was restricted to 24 countries with complete education-stratified SHS exposure data for both years (n = 48 observations). Separately, the regional analysis of variance (ANOVA) was conducted utilizing the same balanced 28-country panel as in the paired samples *t*-tests (n = 56 observations) to evaluate geographical variations. Finally, the education-stratified descriptive analysis was limited to 32 country-level observations for 2019 only, as comparable stratified data were unavailable for 2014.

This method maintains consistency within each statistical process while maximizing the use of available data for each specific analysis. The decision not to include regional grouping in the regression model was made to prevent multicollinearity, as regional context significantly overlaps with education structure and exposure patterns. Regional differences were instead analyzed separately using ANOVA to maintain the interpretability of structural factors at the cross-national level.

Variable definitions

The primary outcome variable is the prevalence of daily smokers (%). The predictor variables are described below. First, education level was categorized according to the International Standard Classification of Education (ISCED) as low (levels 0–2), medium (levels 3–4), and high (levels 5–8). Second, SHS exposure was defined as the percentage of the population exposed to tobacco smoke indoors on a daily basis. Finally, tobacco control policy scores were obtained from the Association of European Cancer Leagues' Tobacco Control Scale (TCS) [20] to assess national legislative strength [21]. The TCS is a composite indicator that evaluates national tobacco control policy based on six criteria: price increases through taxation, smoke-free legislation in public places, budget for public information campaigns, advertising bans, health warning requirements, and cessation treatment availability. Regional groupings were classified into four distinct geographical zones. The Nordic region comprised Denmark, Finland, Iceland, Norway, and Sweden. Western Europe included Austria, Belgium, France, Germany, Ireland, Luxembourg, the Netherlands, and the UK. Southern Europe (SE) encompassed Croatia, Cyprus, Greece, Italy, Malta, Portugal, Serbia, Slovenia, Spain, and Türkiye. Finally, EE consisted of Bulgaria, the Czech Republic, Estonia, Hungary, Latvia, Lithuania, Poland, Romania, and Slovakia.

Statistical analysis

Statistical analyses were performed using SPSS (v26.0). Descriptive statistics are presented as means and standard deviations for continuous variables. To ensure regional representativeness and account for population disparities, European Union (EU) averages were calculated as population-weighted means using the following formula:

$$\overline{x}_w = \frac{\sum_{i=1}^{n}(w_i x_i)}{\sum_{i=1}^{n}(w_i)}$$

Where x_i represents the specific smoking or exposure prevalence rate in country i, and w_i represents the adult population (aged 15+) of that respective country.

Differences in SP between 2014 and 2019 were analyzed using paired samples *t*-tests based on the consistent panel of 28 countries. Regional variations were tested using one-way ANOVA. Finally, a multiple linear regression model was constructed to identify structural predictors of SP, treating country-year observations as the unit of analysis. Model assumptions were evaluated through residual diagnostics, including tests for normality and homoscedasticity. Multicollinearity was checked using variance inflation factors (VIF), with values below 2.0 indicating no signs of mul-

ticollinearity. A *p*-value of < 0.05 was considered statistically significant.

Results

The data presented in Table 1 demonstrate that while the vast majority (75%) of the studied population are non-smokers, a significant segment (20%) consists of daily smokers, the majority of whom consume fewer than 20 cigarettes *per* day. The smoking rate is markedly lower in the highest education group (13%), while it is more prevalent in the medium (22%) and low (19%) education groups. Although the average exposure to indoor tobacco smoke is 11%, this figure rises to as high as 38% in some groups.

Normality tests (Table 2) indicated that the distributions for both the daily smoker and non-smoker variables did not significantly deviate from normality ($p > 0.05$ for both tests). While the Shapiro-Wilk test for SHS exposure indicated a significant deviation ($p = 0.045$), the Kolmogorov-Smirnov test was non-significant ($p = 0.073$). Given the sample size (n = 48), the Central Limit Theorem's applicability, and the Q-Q plot results supporting overall normality, parametric tests were deemed appropriate.

The paired samples *t*-test indicated a statistically significant decrease in the percentage of daily smokers from 2014 (mean = 19.89) to 2019 (mean = 18.98) [$t(55) = 2.341$, $p = 0.023$]. Conversely, there was a statistically significant increase in the percentage of non-smokers over the same period [$t(55) = -2.156$, $p = 0.036$] (Table 3).

The results of the ANOVA for daily SP by educational attainment are shown in Table 4, demonstrating statistically significant differences in DS rates among education levels.

There was no significant difference between the low and medium education groups ($p = 0.183$) (Table 5). Comparing low vs. high education groups revealed a significant difference ($p < 0.001$), with a higher prevalence in the low education group. Similarly, a significant difference was found between medium and high education groups ($p < 0.001$), with the medium education group exhibiting higher SP.

Descriptive statistics for daily SP stratified by European region are presented in Table 6a.

A one-way ANOVA (Table 6b) was conducted to compare the differences across regions on DS prevalence. There was a statistically significant difference in smoking rates between at least two regions [$F(3, 52) = 6.11$, $p < 0.001$]. The

Table 2

Tests of normality for primary outcome and predictor variables

Variable	Kolmogorov-Smirnov test	*p*-value	Shapiro-Wilk test	*p*-value
Daily smoker	0.064	0.200	0.978	0.089
Non-smoker	0.058	0.200	0.984	0.234
Secondhand smoke	0.078	0.073	0.967	0.045

Table 3

Paired samples *t*-test comparing national prevalence rates of daily smoking and non-smoking between 2014 and 2019

Variable	Mean ± SD		SE mean		*t*-test	*df*	*p*-value	MD
	2014	2019	2014	2019				
Daily smoker	19.89 ± 5.67	18.98 ± 5.94	0.76	0.70	2.341	55	0.023	0.91
Non-smoker	74.98 ± 5.21	75.68 ± 5.29	0.79	0.71	-2.156	55	0.036	-0.70

SD – standard deviation; SE – standard error; *df* – degrees of freedom; MD – mean difference.

Table 4

One-way analysis of variance (ANOVA) of daily smoking prevalence by educational attainment

Source	Sum of squares	*df*	Mean square	*F*	*p*-value
Between groups	1,156.34	2	578.17	15.89	0.000
Within groups	3,278.45	93	36.38		
Total	4,434.79	95			

***df* – degrees of freedom.**
***Note: F* (2, 93) = 15.89, *p* < 0.001.**

Table 5

***Post hoc* comparisons – Tukey HSD test of daily smoking prevalence by educational attainment**

(I) Education	(J) Education	MD (I-J)	SE	*p*-value	95% CI
Low	Medium	-2.61	1.51	0.183	-6.12–0.90
	High	5.83	1.51	0.000	2.32–9.34
Medium	High	8.44	1.51	0.000	4.93–11.95

HSD – honestly significant difference; MD – mean difference; SE – standard error; CI – confidence interval.
***Note:* I and J stand for MD between groups I and J.**

mean scores show a clear gradient, with the lowest SP in Nordic countries (12.35%) and the highest in EE countries (22.89%).

As shown in Table 7, the correlation reveals several significant relationships. There is a very strong negative correlation between daily smoker and non-smoker rates (r = -0.95, $p < 0.001$). DS is positively correlated with SHS exposure (r = 0.456, $p < 0.001$) and negatively correlated with education level (r = -0.401, $p < 0.001$), indicating that higher education is associated with lower smoking rates. The significant negative coefficient for education level (β = -0.401, $p < 0.001$) indicates that as the education level increases from low (coded as 1) to high (coded as 3), the predicted prevalence of DS decreases.

A multiple regression was conducted to predict DS percentage from SHS exposure, education level, and year (Table 8). The model was statistically significant [F(3, 58) = 22.37, $p < 0.001$] and explained 51.2% of the variance (R^2 = 0.512). All three variables emerged as significant predictors: SHS exposure (β = 0.367, $p < 0.001$), education level (β = -0.401, $p < 0.001$), and year (β = -0.167, p = 0.037). These findings confirm that higher SHS exposure predicts higher smoking rates, higher education attainment predicts lower smoking rates, and smoking rates were significantly lower in

Table 6a

Descriptive statistics for daily smoking prevalence stratified by European region

Region	Mean ± SD	SE	95% CI
Nordic countries (n = 8)	12.35 ± 3.45	1.22	9.42–15.28
Western Europe (n = 16)	18.92 ± 4.78	1.20	16.35–21.49
Southern Europe (n = 12)	20.56 ± 5.23	1.51	17.23–23.89
Eastern Europe (n = 20)	22.89 ± 4.12	0.92	20.96–24.82
Total (n = 56)	19.24 ± 5.87	0.78	17.66–20.82

SD – standard deviation; SE – standard error; CI – confidence interval.
Note: **n indicates the number of country–year observations in each group. The analysis includes data from 28 countries across two time points (2014 and 2019), yielding 56 country-year observations.**

Table 6b

One-way analysis of variance (ANOVA) results for differences in daily smoking prevalence across European regions

Source	Sum of squares	*df*	Mean square	*F*	*p*-value
Between groups	2,156.78	3	718.93	6.11	< 0.001
Within groups	6,123.45	52	117.76		
Total	8,280.23	55			

***df* – degrees of freedom.**

Table 7

Bivariate Pearson correlations among key study variables

Variable	Daily smoker	Non-smoker	Indoor tobacco smoke exposure	Electronic cigarette	Education level
Daily smoker	1.000				
Non-smoker	-0.95***	1.000			
Indoor exposure	0.456***	-0.389***	1.000		
E-cigarette	0.234*	-0.198	0.312**	1.000	
Education level	-0.401***	0.367***	-0.289**	-0.156	1.000

****** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.***

Table 8

Multiple linear regression model predicting daily smoking prevalence (2014–2019)

Variable	Standardized coefficients (β)	*p*-value
Indoor tobacco smoke exposure	0.367	< 0.001
Education level	-0.401	< 0.001
Year (2019)	-0.167	0.037
Model summary	R^2 = 0.512; F(3, 58) = 22.37	< 0.001

Dependent variable: daily smoking prevalence (%).
Year reference category: 2014. Education level: ordinal (1 = low to 3 = high). Supplementary model parameters: indoor exposure: B = 0.241, standard error (SE) = 0.058, 95% confidence interval (CI) (0.125, 0.357), variance inflation factor (VIF) = 1.42; education level: B = -2.18, SE = 0.46, 95% CI (-3.11, -1.25), VIF = 1.31; year (2019): B = -0.91, SE = 0.43, 95% CI (-1.77, -0.05), VIF = 1.09. All VIF values < 2.0, indicating no multicollinearity concerns.

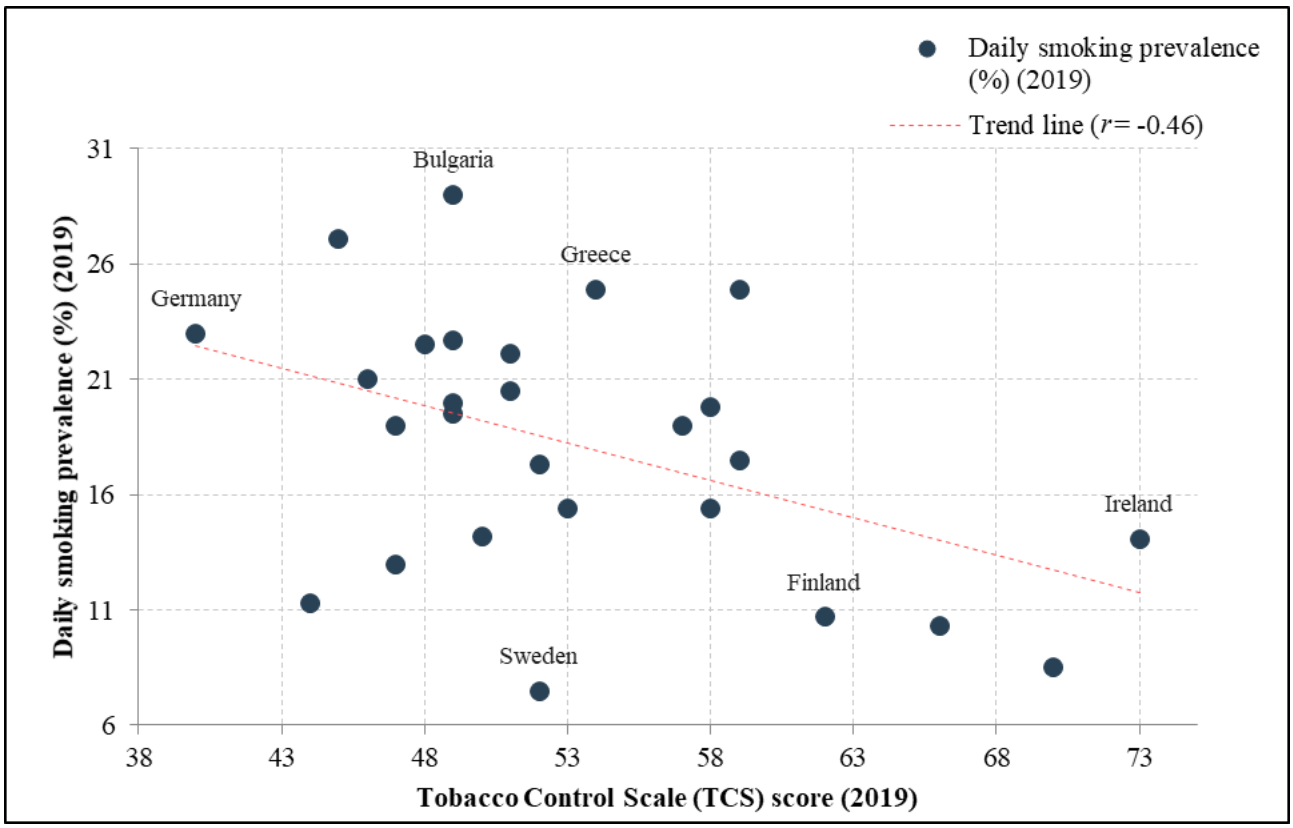

Fig. 1 – Correlation between TCS scores and daily smoking prevalence (2019). Higher TCS scores are associated with lower smoking rates (r = -0.46, p < 0.05). Countries with stricter policies (e.g., Ireland) have significantly lower prevalence compared to those with weaker enforcement (e.g., Germany, Bulgaria). Sources: Eurostat [19] and Joossens and Raw (2020) [20].

2019 compared to 2014. Full model parameters, including unstandardized coefficients, standard errors, and 95% confidence intervals, are detailed in Table 8. Multicollinearity was assessed using VIF; all VIF values were below 2.0 (SHS: VIF = 1.42; education level: VIF = 1.31; year: VIF = 1.09), indicating no problematic multicollinearity. Model assumptions were verified: residuals were approximately normally distributed (Shapiro-Wilk p = 0.18) and homoscedastic (Levene's test p = 0.21).

Although the TCS is utilized descriptively to contextualize cross-national policy variations (Figure 1) [19], it was deliberately excluded as a predictor in the multivariate regression model for two distinct methodological reasons. First, TCS scores were available only for 2019, precluding the use of TCS as a time-varying predictor across the longitudinal sample (n = 62 observations). Second, given that TCS is structurally correlated with both regional affiliation and educational attainment, its inclusion would introduce multicollinearity, thereby risking the distortion of the independent contributions of education and SHS exposure that the model was designed to quantify. Consequently, the association between TCS and overall SP is examined solely through the visual and descriptive analysis presented in Figure 1.

A detailed country comparison presented in Table 9 identifies Türkiye's persistently high SP as a particular concern. In Türkiye, DS remained stagnant at around 28% between 2014 and 2019, and a deeply entrenched 14.8% of the population was classified as heavy smokers. This stagnation, combined with a substantial increase in indoor SHS exposure from 14.2% to 16.7%, demonstrates a sharp divergence from nations with aggressive tobacco control policies. Furthermore, Türkiye exhibited one of the lowest smoking cessation rates in Europe; its former smoker rate stood at just 17.5%, surpassing only Cyprus (17.4%), Greece (15.7%), and Romania (10.7%). The situation stands in sharp contrast to positive trends in countries like Greece, which saw significant reductions in smoking and SHS exposure, highlighting a critical need for Türkiye to strengthen smoke-free policy enforcement and enhance cessation services to avert rising tobacco-related diseases.

Regional disparities and the 'East-West' divide

A stark regional divide persists. While Nordic countries such as Sweden (7.4%) and Finland (10.7%) reached tobacco endgame prevalence levels in 2019, EE and SE nations faced stagnation. Türkiye, for instance, showed no meaningful change in daily SP between 2014 (27.3%) and 2019 (28.0%). Of greater concern, the intensity of smoking in Türkiye remains high; in 2019, 14.8% of the population consumed 20 or more cigarettes daily─a rate unchanged from 2014 and more than double the EU average of approximately 6%. Similarly, Bulgaria reported a slight increase

Table 9

Tobacco use comparison: Türkiye vs. EU average and selected reference countries representing high (Bulgaria, Greece) and low (Sweden) prevalence rates (2014–2019)

Country	Daily smoker	Non-smoker	20+ cig/day	Indoor SHS exposure	Former smoker
Türkiye					
2014	27.3	67.5	14.8	14.2	-
2019	28.0	68.6	14.8	16.7	17.5
EU average					
2014	19.2	76.1	5.8	10.8	-
2019	19.3	75.8	5.9	10.3	30.7
Bulgaria					
2014	28.2	65.2	12.7	20.0	-
2019	29.1	63.8	12.9	17.1	19.7
Greece					
2014	27.3	67.4	15.1	37.9	-
2019	24.9	71.4	10.8	19.4	15.7
Germany					
2014	15.9	78.3	5.0	7.7	-
2019	22.9	71.7	7.8	9.9	-
France					
2014	22.4	71.7	4.6	11.3	-
2019	18.5	76.0	4.1	7.8	30.9
Sweden					
2014	9.8	83.3	1.2	2.3	-
2019	7.4	87.4	1.0	2.6	32.5

EU – European Union; Cig – cigarettes; SHS – secondhand smoke.
Values are given in percentages.
Note: **All 'EU average' values presented in this table were calculated as population-weighted means, consistent with the data aggregation method described in the Methods section. Former smoker data for Germany were not available for the years studied.**

in DS to 29.1%. These findings indicate heterogeneity in smoking patterns across European regions and suggest persistent differences in tobacco use intensity and prevalence between countries.

The environmental factor (indoor exposure)

SHS exposure data indicates gaps in the implementation or enforcement of protection policies in specific regions. While daily SHS exposure in Finland remained low at approximately 2%, it increased significantly in Türkiye from 14.2% (2014) to 16.7% (2019). High exposure rates were also observed in Greece (19.4%) and Croatia (26.1%) in 2019. This correlates strongly with overall prevalence, suggesting that non-enforcement of smoke-free laws in private and semi-private spaces creates an "environmental lock-in" that may hinder cessation.

Correlation with Tobacco Control Scale scores

To complement the statistical analysis, we visualized the relationship between policy strictness and prevalence directly (Figure 1). The analysis reveals a consistent inverse pattern between TCS scores and SP across countries. Countries with high TCS scores, such as Ireland (score: 73) and the UK, have successfully suppressed smoking rates to below 15%. Conversely, nations with lower scores, including Germany (score: 40) and Bulgaria (score: 48), continue to struggle with high prevalence (> 22%). These patterns are consistent with the hypothesis that differences in the strength and enforcement of tobacco control policies contribute to observed cross-national variation in SP. However, given the ecological design of the study, these associations should be interpreted as descriptive and hypothesis-generating rather than causal.

Discussion

This study provides a comprehensive, integrated analysis of the multilevel determinants of SP across a diverse set of nations. The results powerfully illustrate that smoking is not merely an individual behavior but a socio-ecological outcome, deeply embedded within educational structures, environmental contexts, and regional policy landscapes. The profound educational gradient observed aligns strongly with the theory of "fundamental causes" of health inequalities [6, 22]. The 8.44 percentage point gap between the medium and high education groups is a stark manifestation of how socioeconomic resources translate into health behaviors. Countries with higher educational attainment tend to show lower aggregate SP, consistent with evidence that higher education is associated with greater health literacy, future-oriented thinking, and access to social environments where healthy choices are normative and supported [8, 23]. The absence of a significant difference between the low and medium education groups is a critical nuance, suggesting that the most powerful protective effect of education may operate as a threshold, likely

at the tertiary level. This finding resonates with studies indicating that university education, in particular, imparts critical cognitive skills and social networks that are highly effective in mitigating health-risk behaviors [24]. The stark regional gradient from Nordic to EE countries provides a vivid illustration of the impact of policy path dependency and political commitment. The success of Nordic countries is not accidental; it is the result of a long-term, consistently applied, and comprehensive strategy encompassing high taxation, extensive smoke-free laws, plain packaging, and public education campaigns [15, 24, 25]. Conversely, the high prevalence in EE countries has been linked to the historical influence of the tobacco industry, slower transposition of EU tobacco directives into national law, and weaker enforcement mechanisms [16, 26]. This regional pattern underscores that national and supra-national policy environments create a "risk regime" that powerfully shapes population-level health outcomes [27]. Given the ecological nature of this analysis, however, these country-level associations should not be interpreted as reflecting direct causal mechanisms at the individual level. The public health significance of these findings for EE countries is substantial. The mean daily SP of 22.89% observed in this region substantially exceeds estimates reported in prior comparative studies of Central European and EE populations. Those earlier investigations documented rates in the range of 20–25% during the same period, noting that the burden was heavily concentrated among lower-educated and economically disadvantaged groups [16, 26]. Critically, our data indicate that this elevated prevalence is not merely a historical legacy but an active, ongoing phenomenon: Bulgaria recorded an increase from 28.2% to 29.1% between 2014 and 2019, while no meaningful reduction was observed across the EE sub-region as a whole. These findings are consistent with documentation by Schaap et al. [16] that nationwide tobacco control policies have disproportionately benefited higher-educated groups in Europe, leaving low-SES populations in structurally disadvantaged regions with fewer gains. Taken together, the evidence points to a widening intra-European health equity gap that demands region-specific policy responses: accelerated transposition of EU tobacco directives, targeted cessation programs for low-education populations, and strengthened cross-border enforcement coordination within EE member states. Our analysis utilizes TCS, a quantitative instrument that evaluates the implementation of tobacco control policies across European countries based on six criteria: price increases, smoke-free public places, budget for information campaigns, advertising bans, health warnings, and cessation treatment availability. The strong negative correlation between TCS scores and prevalence confirms that comprehensive legislative frameworks are essential for reducing smoking rates [21]. As noted in the Methods section, TCS was examined descriptively rather than incorporated into the regression model, due to the availability of its scores for 2019 only and the risk of multicollinearity with regional and educational variables. The patterns observed in Figure 1 are therefore presented as hypothesis-generating. The significant role of SHS exposure reinforces its dual nature as both a consequence and a cause of SP. Its persistence as a strong predictor in the multivariate model indicates that it exerts an independent environmental influence, likely by reinforcing the social normalcy of smoking and creating cues that trigger relapse among quitters [11, 28]. This finding provides a robust evidence base for the continued expansion and stringent enforcement of smoke-free legislation beyond public spaces to include semi-private areas like multi-unit housing and vehicles, as recommended by leading public health bodies [29]. Our updated data analysis also reveals alarming trends in non-EU SE countries. For instance, in Serbia, daily SHS exposure to tobacco smoke was recorded at 33.9% in 2019, a rate significantly higher than the EU average and even exceeding that of Greece. This suggests a critical regional gap in the enforcement of smoke-free legislation in the Balkans [19]. The modest but significant temporal decline in smoking, net of other factors, is consistent with global reports and reflects the cumulative impact of decades of tobacco control efforts [1, 2]. Our regression model confirmed this trend, showing that SP in 2019 was significantly lower than in the 2014 baseline, even after controlling for education levels and SHS exposure. While the overall trend points to a decline, this pattern was not uniform across all nations, underscoring the uneven nature of progress in tobacco control. A notable deviation from the general decline was observed in Germany, where daily SP rose from 15.9% in 2014 to 22.9% in 2019. While methodological integration of the Microcensus in German survey systems in 2019 may account for some statistical noise, this sharp increase cannot be dismissed merely as an artifact. Rather, it aligns with Germany's historically low ranking on TCS, which is characterized by weaker tobacco advertising bans and lower taxation compared to its Western European neighbors. This suggests that without stringent policy enforcement, economic development alone is insufficient to curb smoking rates. The observed increase in SP in Germany warrants a nuanced interpretation. Beyond methodological changes in the Microcensus, sociodemographic factors likely play a role. Germany hosts a significant population of immigrants from high-prevalence regions, including EE countries and Türkiye. Studies suggest that acculturation processes can be complex; while some immigrant groups adopt the healthier behaviors of the host country, first-generation immigrants often retain the higher SP typical of their countries of origin, which correlates with lower SES. This demographic composition, combined with Germany's comparatively lenient tobacco control policies, creates a specific vulnerability [30]. This outlier serves as a critical reminder that aggregate progress can mask significant setbacks in individual countries, and it highlights the necessity for continuous monitoring and context-specific policy evaluations to understand and reverse such adverse trends. It should be noted that former smoker data for Germany were not available in the dataset for the years studied, limiting a more complete analysis of smoking cessation dynamics in this specific case. Crucially, while the associa-

tion between SES and smoking is established, our findings highlight a critical "hardening" of the landscape. The divergence between nations like Sweden and Türkiye suggests that the European region is moving at two different speeds. Unlike trends observed in countries such as Poland (4.6% daily use) or Iceland (4.1%), daily e-cigarette use in Türkiye remains negligible at 0.4% [19]. This suggests that the stagnation in SP is not due to a substitution effect towards novel products, but rather a persistent reliance on combustible tobacco. The debate regarding novel products remains complex; while some evidence suggests potential for harm reduction, the concurrent use of combustible tobacco and e-cigarettes undermines cessation efforts, particularly in environments with weak regulatory enforcement [31]. While cessation rates in the Nordic countries show strong socioeconomic gradients, Türkiye exhibits consistently low cessation rates across all income quintiles [19]. This suggests limited reach or effectiveness of cessation support services across socioeconomic boundaries, contrasting with the targeted success seen in Nordic regions. The stagnation in high-prevalence countries, coupled with rising SHS exposure rates in countries like Türkiye, indicates that current legislative frameworks have limited influence on private social norms. This supports the hypothesis that we have reached the limits of "generic" tobacco control. Recent global analyses affirm that while standard policies like taxation have driven past successes, their impact is plateauing in high-prevalence demographic pockets, necessitating more targeted, equity-focused strategies [32]. The remaining disparity is structural and requires targeted enforcement rather than broad education campaigns. This group is marked by intense nicotine addiction, a lack of response to standard cessation messages, and a significant overrepresentation among lower SES groups. These characteristics render future public health efforts substantially more challenging [33, 34]. This underscores the imperative to move beyond generic campaigns to more intensive, targeted cessation support. The findings collectively argue for a strategic evolution in tobacco control, from a primarily universal approach to one that explicitly prioritizes equity. Addressing the educational gap requires upstream interventions that begin early in the life course. Strengthening educational systems and improving graduation rates, particularly at the secondary and tertiary levels, can itself be a powerful tobacco control strategy [35]. Cessation services must be proactively integrated into settings that serve low-SES populations, such as vocational training centers, social service agencies, and community health clinics. These services should be low-cost or free, easily accessible, and culturally tailored [36, 37]. The fight for comprehensive smoke-free laws must continue, requiring vigilance against industry-backed exemptions. Public health advocacy should focus on closing loopholes and extending protections to all indoor and some outdoor public spaces [14]. The regional disparities call for enhanced technical and financial support for policy adoption in high-prevalence regions. Mechanisms for policy learning and collaboration, facilitated by bodies such as the WHO and the EU, are essential to accelerate progress in lagging regions [38]. Moving forward, adopting a tobacco endgame strategy–aiming for less than 5% prevalence–requires not only structural changes but also strict regulation of new nicotine delivery systems to prevent a new generation of addiction [39].

Limitation of the study

This study has several limitations. First, the analysis relies on country-level aggregated data, which precludes individual-level inference and risks the ecological fallacy. Due to the ecological design, the associations observed between education, SHS exposure, and SP at the country level cannot be interpreted as causal relationships at the individual level. Country-level correlations may not reflect individual-level associations, and unmeasured confounding at the country level remains possible. Second, while the three-tier International Standard Classification of Education (ISCED) framework and its ordinal coding were operationalized appropriately, this broad measure may not capture critical nuances in educational quality, specific attainment thresholds, or the specific mechanisms linking education to health behaviors. Third, despite the model's substantial explanatory power ($R^2 = 0.512$), it omits other potent determinants of smoking, such as tobacco taxation, marketing exposure, and mental health factors. The measure of SHS exposure, though a significant predictor, is based on self-report and may be susceptible to bias. Fourth, our multivariate regression model was designed to identify the independent predictive power of key socio-economic and environmental factors—education and SHS exposure on SP. While regional differences were a central descriptive finding of our study, the 'Region' variable was not included in the final regression model. This decision was made to avoid potential multicollinearity, as educational attainment and smoke exposure are often structurally embedded within regional contexts. Including region risked obscuring the specific, cross-national effects of education and environment that we aimed to quantify. Similarly, TCS was not included in the regression model due to the availability of scores only for 2019 and the structural overlap with both regional affiliation and educational attainment, which would have introduced multicollinearity. Its role was therefore examined descriptively. Finally, data on novel nicotine products (e.g., e-cigarettes) were too limited for a thorough analysis of their role in smoking disparities, representing an important avenue for future research.

Conclusion

This multinational study confirms that while overall smoking prevalence has declined, profound inequalities based on education, region, and environmental exposure persist. At the country level, lower educational attainment and widespread indoor secondhand smoke exposure are associated with higher smoking rates. Current tobacco control efforts have not yet fully mitigated these disparities. Future progress requires a strategic shift from blanket ap-

proaches to an equity-oriented framework that explicitly prioritizes low-socioeconomic status populations, implements stringent smoke-free policies, and fosters cross-regional policy learning. Given the ecological nature of this study, these findings should be interpreted as descriptive and hypothesis-generating; future individual-level and multilevel analyses are warranted to further elucidate the mechanisms underlying the observed associations.

Conflicts of interest

The authors declare no conflict of interest.

Funding

The authors received no financial support for the research, authorship, and/or publication of this article.

R E F E R E N C E S

1. *World Health Organization.* WHO global report on trends in prevalence of tobacco use 2000-2025, 4th edition [Internet]. Geneva: WHO; 2021 [cited 2026 May 21]. Available from: https://www.who.int/publications/i/item/9789240039322
2. *Gravely S, Giovino GA, Craig L, Commar A, D'Espaignet ET, Schotte K,* et al. Implementation of key demand-reduction measures of the WHO Framework Convention on Tobacco Control and change in smoking prevalence in 126 countries: an association study. Lancet Pub Health 2017; 2(4): e166–74. DOI: 10.1016/S2468-2667(17)30045-2.
3. *Giskes K, Kunst AE, Benach J, Borrell C, Costa G, Dahl E,* et al. Trends in smoking behaviour between 1985 and 2000 in nine European countries by education. J Epidemiol Commun Health 2005; 59(5): 395–401. DOI: 10.1136/jech.2004.025684.
4. *Harper S, Lynch J.* Trends in socioeconomic inequalities in adult health behaviors among US states, 1990–2004. Public Health Rep 2007; 122(2): 177–89. DOI: 10.1177/003335490712200207.
5. *Hiscock R, Bauld L, Amos A, Fidler JA, Munafò M.* Socioeconomic status and smoking: a review. Ann N Y Acad Sci 2012; 1248(1): 107–23. DOI: 10.1111/j.1749-6632.2011.06202.x.
6. *Phelan JC, Link BG, Tehranifar P.* Social conditions as fundamental causes of health inequalities: theory, evidence, and policy implications. J Health Soc Behav 2010; 51 Suppl: S28–40. DOI: 10.1177/0022146510383498.
7. *Stringhini S, Sabia S, Shipley M, Brunner E, Nabi H, Kivimaki M,* et al. Association of socioeconomic position with health behaviors and mortality. JAMA 2010; 303(12): 1159–66. DOI: 10.1001/jama.2010.297.
8. *Cutler DM, Lleras-Muney A.* Understanding differences in health behaviors by education. J Health Econ 2010; 29(1): 1–28. DOI: 10.1016/j.jhealeco.2009.10.003.
9. *Siahpush M, Singh GK, Jones PR, Timsina LR.* Racial/ethnic and socioeconomic variations in duration of smoking: results from 2003, 2006 and 2007 Tobacco Use Supplement of the Current Population Survey. J Pub Health (Oxf) 2010; 32(2): 210–8. DOI: 10.1093/pubmed/fdp104.
10. *Brown T, Platt S, Amos A.* Equity impact of interventions and policies to reduce smoking in youth: systematic review. Tob Control 2014; 23(e2): e98–105. DOI: 10.1136/tobaccocontrol-2013-051451.
11. *Akhtar PC, Haw SJ, Levin KA, Currie DB, Zachary R, Currie CE.* Socioeconomic differences in second-hand smoke exposure among children in Scotland after introduction of the smoke-free legislation. J Epidemiol Commun Health 2010; 64(4): 341–6. DOI:10.1136/jech.2008.084178.
12. *Mons U, Nagelhout GE, Allwright S, Guignard R, van den Putte B, Willemsen MC,* et al. Impact of national smoke-free legislation on home smoking bans: findings from the International Tobacco Control Policy Evaluation Project Europe Surveys. Tob Control 2013; 22(e1): e2–9. DOI: 10.1136/tobaccocontrol-2011-050131.
13. *Frazer K, Callinan JE, McHugh J, van Baarsel S, Clarke A, Doherty K,* et al. Legislative smoking bans for reducing harms from secondhand smoke exposure, smoking prevalence and tobacco consumption. Cochrane Database Syst Rev 2016; 2(2): CD005992. DOI: 10.1002/14651858.CD005992.pub3.
14. *Nagelhout GE, Hummel K, Willemsen MC, Siahpush M, Kunst AE, de Vries H,* et al. Are there income differences in the impact of a national reimbursement policy for smoking cessation treatment and accompanying media attention? Findings from the International Tobacco Control (ITC) Netherlands Survey. Drug Alcohol Depend 2014; 140: 183–90. DOI: 10.1016/j.drugalcdep.2014.04.012.
15. *Gilmore AB, Fooks G, Drope J, Bialous SA, Jackson RR.* Exposing and addressing tobacco industry conduct in low-income and middle-income countries. Lancet 2015; 385(9972): 1029–43. DOI: 10.1016/S0140-6736(15)60312-9.
16. *Schaap MM, Kunst AE, Leinsalu M, Regidor E, Ekholm O, Dzurova D,* et al. Effect of nationwide tobacco control policies on smoking cessation in high and low educated groups in 18 European countries. Tob Control 2008; 17(4): 248–55. DOI: 10.1136/tc.2007.024265.
17. *Hill S, Amos A, Clifford D, Platt S.* Impact of tobacco control interventions on socioeconomic inequalities in smoking: review of the evidence. Tob Control 2014; 23(e2): e89–97. DOI: 10.1136/tobaccocontrol-2013-051110.
18. *Diez-Roux AV.* Multilevel analysis in public health research. Annu Rev Public Health 2000; 21: 171–92. DOI: 10.1146/annurev.publhealth.21.1.171.
19. *EUROSTAT.* Database [Internet]. Luxembourg: EUROSTAT; 2025 [cited 2025 Nov 15; accessed 2026 May 21]. Available from: https://ec.europa.eu/eurostat/data/database
20. *Joossens L, Raw M.* The Tobacco Control Scale: a new scale to measure country activity. Tob Control 2006; 15(3): 247-53. DOI: 10.1136/tc.2005.015347.
21. *Joossens L, Feliu A, Fernandez E.* The Tobacco Control Scale 2019 in Europe [Internet]. Brussels: Association of European Cancer Leagues, Catalan Institute of Oncology; 2020 [cited on 2026 May 21]. Available from: https://www.tobaccocontrolscale.org/TCS2019.pdf
22. *Link BG, Phelan J.* Social conditions as fundamental causes of disease. J Health Soc Behav 1995; Spec No: 80–94. DOI: 10.2307/2137284.
23. *Mirowsky J, Ross CE.* Education, health, and the default American lifestyle. J Health Soc Behav 2015; 56(3): 297–306. DOI: 10.1177/0022146515594814.
24. *Lawrence EM.* Why do college graduates behave more healthfully than those who are less educated? J Health Soc Behav 2017; 58(3): 291–306. DOI: 10.1177/0022146517715671.
25. *Fosse E, Helgesen MK, Hagen S, Torp S.* Addressing the social determinants of health at the local level: Opportunities and challenges. Scand J Public Health 2018; 46(20_suppl): 47–52. DOI: 10.1177/1403494817743896.
26. *Stuckler D, McKee M, Ebrahim S, Basu S.* Manufacturing epidemics: the role of global producers in increased consumption of unhealthy commodities including processed foods, alcohol, and tobacco. PLoS Med 2012; 9(6): e1001235. DOI: 10.1371/journal.pmed.1001235.

27. *Beckfield J, Krieger N.* Epi+ demos+ cracy: linking political systems and priorities to the magnitude of health inequities—evidence, gaps, and a research agenda. Epidemiol
28. *Fong GT, Hyland A, Borland R, Hammond D, Hastings G, McNeill A,* et al. Reductions in tobacco smoke pollution and increases in support for smoke-free public places following the implementation of comprehensive smoke-free workplace legislation in the Republic of Ireland: findings from the ITC Ireland/UK Survey. Tob Control 2006; 15(suppl 3): iii51–8. DOI: 10.1136/tc.2005.013649.
29. *U.S. Department of Health and Human Services.* The health consequences of smoking—50 years of progress: a report of the Surgeon General [Internet]. Atlanta (GA): U.S. Department of Health and Human Services; 2014 [cited 2026 May 21]. Available from: https://www.hhs.gov/sites/default/files/consequences-smoking-exec-summary.pdf
30. *Reiss K, Spallek J, Razum O.* Effect of length of stay on smoking among Turkish and Eastern European immigrants in Germany—Interpretation in the light of the smoking epidemic model and the acculturation theory. Int J Environ Res Public Health 2015; 12(12): 15925–36. DOI: 10.3390/ijerph121215030.
31. *Balfour DJ, Benowitz NL, Colby SM, Hatsukami DK, Lando HA, Leischow SJ,* et al. Balancing Consideration of the Risks and Benefits of E-Cigarettes. Am J Public Health 2021; 111(9): 1661–72. DOI: 10.2105/AJPH.2021.306416. Erratum in: Am J Public Health 2022; 112(4): e6. DOI: 10.2105/AJPH.2022.306829.
32. *Flor LS, Reitsma MB, Gupta V, Ng M, Gakidou E.* The effects of tobacco control policies on global smoking prevalence. Nat Med 2021; 27(2): 239–43. DOI: 10.1038/s41591-020-01210-8.
33. *Warner KE, Burns DM.* Hardening and the hard-core smoker: concepts, evidence, and implications. Nicotine Tob Res 2003; 5(1): 37–48. DOI: 10.1080/1462220021000060428.
34. *Kulik MC, Glantz SA.* The smoking population in the USA and EU is softening not hardening. Tob Control 2016; 25(4): 470–5. DOI: 10.1136/tobaccocontrol-2015-052329.
35. *Vuolo M, Staff J.* Parent and child cigarette use: a longitudinal, multigenerational study. Pediatrics 2013; 132(3): e568–77. DOI: 10.1542/peds.2013-0067.
36. *Bryant J, Bonevski B, Paul C, McElduff P, Attia J.* A systematic review and meta-analysis of the effectiveness of behavioural smoking cessation interventions in selected disadvantaged groups. Addiction 2011; 106(9): 1568–85. DOI: 10.1111/j.1360-0443.2011.03467.x.
37. *U.S. Department of Health and Human Services.* Smoking cessation: a report of the Surgeon General[Internet]. Atlanta (GA): U.S. Department of Health and Human Services; 2020 [cited 2026 May 21]. Available from: https://www.hhs.gov/sites/default/files/2020-cessation-sgr-full-report.pdf
38. *Smith KE, Fooks G, Collin J, Weishaar H, Mandal S, Gilmore AB.* "Working the system"—British American tobacco's influence on the European union treaty and its implications for policy: an analysis of internal tobacco industry documents. PLoS Med 2010; 7(1): e1000202. DOI: 10.1371/journal.pmed.1000202.
39. *Beaglehole R, Bonita R.* Tobacco control: getting to the finish line. The Lancet 2022; 399(10338): 1865. DOI: 10.1016/S0140-6736(22)00835-2.

Received on December 10, 2025
Revised on May 5, 2026
Revised on May 8, 2026
Revised on May 20, 2026
Accepted on May 27, 2026
Online First July 2026

ORIGINAL ARTICLE

UDC: 618.11-006:616.89-008.454]:577.161.2
DOI: https://doi.org/10.2298/VSP250925035S

Relationship between vitamin D levels and depression in women with polycystic ovary syndrome

Povezanost između nivoa vitamina D i depresije kod žena koje imaju sindrom policističnih jajnika

Emine Sevinc Postaci*, Suat Cakina*, Fatma Beyazit†

Çanakkale Onsekiz Mart University, †Faculty of Medicine, Department of Obstetrics and Gynecology, *Health Service Vocational College, Çanakkale, Türkiye

Abstract

Background/Aim. Polycystic ovary syndrome (PCOS) is a common gynecological disorder in women of reproductive age. Depression is a common PCOS-related comorbidity that affects women's mental state. Although vitamin D deficiency (VDD) has been associated with affective dysregulation through immuno-inflammatory and neuroendocrine pathways, evidence in PCOS remains limited. The aim of this study was to determine the relationship between serum 25-hydroxyvitamin D [25(OH)D] deficiency and Beck Depression Inventory (BDI) scores in women with PCOS. **Methods.** This cross-sectional study included 80 women with PCOS who completed the BDI on the same day when their fasting serum 25(OH)D levels were measured. Multivariable models were prespecified to compare depressive symptom (DS) severity across vitamin D status, adjusting for key confounders such as age, body mass index, season, and insulin resistance. **Results.** Women with VDD experienced a greater burden of DSs than women without VDD ($p < 0.05$). This association persisted after adjusting for covariates in sensitivity analyses. **Conclusion.** Among women with PCOS, VDD was associated with greater DS severity. However, causal relationships cannot be established, and further longitudinal and interventional studies are needed.

Keywords:
depression; polycystic ovary syndrome; surveys and questionnaires; turkish people; vitamin d deficiency.

Apstrakt

Uvod/Cilj. Sindrom policističnih jajnika (*polycystic ovary syndrome* – PCOS) je čest ginekološki poremećaj kod žena u reproduktivnom dobu. Depresija je čest komorbiditet povezan sa PCOS-om koji utiče na mentalno stanje žena. Iako se nedostatak vitamina D (vitamin D *deficiency* – VDD) povezuje sa afektivnom disregulacijom posredovanom mehanizmima imunsko-inflamacijskih i neuroendokrinih puteva, dokazi o ovoj povezanosti kod PCOS i dalje su oskudni. Cilj ove studije bio je da se utvrdi odnos između nedostatka 25-hidroksivitamina D [25(OH)D] u serumu i rezultata Bekove skale depresivnosti (*Beck Depression Inventory* – BDI) kod žena koje imaju PCOS. **Metode.** Studijom preseka obuhvaćeno je 80 žena sa PCOS koje su popunile BDI istog dana kada im je natašte izmeren nivo 25(OH)D u serumu. Multivarijantni modeli određeni su unapred da bi se uporedila težina simptoma depresije u odnosu na status vitamina D, uz prilagođavanje za ključne zbunjujuće faktore kao što su životno doba, indeks telesne mase, godišnje doba i insulinska rezistencija. **Rezultati**. Žene sa VDD imale su značajno veće opterećenje simptomima depresije u odnosu na žene bez VDD ($p < 0,05$). Ta povezanost ostala je prisutna i nakon prilagođavanja kovarijablama u analizama osetljivosti. **Zaključak.** Kod žena koje imaju PCOS, VDD je bio povezan sa težim stepenom simptoma depresije. Međutim, ne mogu se utvrditi uzročno-posledične veze i potrebna su dalja longitudinalna i interventna istraživanja.

Ključne reči:
depresija; jajnik, policistični, sindrom; ankete i upitnici; turska; vitamin d, nedostatak.

Introduction

Polycystic ovary syndrome (PCOS) is a common endocrine and metabolic disorder that affects reproduction, metabolism, and mental health in women of reproductive age worldwide [1, 2]. Individuals with PCOS are more likely to experience significant anxiety and depressive symptoms (DSs) than those without the condition. Women with PCOS are particularly vulnerable to anxiety and depression during the transition from adolescence to adulthood [3, 4].

Correspondence to: Emine Sevinc Postaci, Health Service Vocational College, Çanakkale Onsekiz Mart University, Çanakkale, Türkiye. E-mail: emnsvnc@comu.edu.tr

Vitamin D (VD) deficiency (VDD) is widespread in countries with significant seasonal and latitudinal variability, such as Türkiye, and has been associated with increased risk of anxiety and DSs [5, 6]. VD plays a role in neurobiological processes that influence depression by regulating proinflammatory cytokines, connecting with the hypothalamic–pituitary-adrenal axis, and modulating serotonergic pathways by controlling the tryptophan hydroxylase gene. PCOS is a disorder characterized by obesity, insulin resistance, and chronic low-grade inflammation, all of which may influence these pathways [7–9].

Studies have shown that low 25-hydroxyvitamin D [25(OH)D] levels are associated with an increased risk of DSs. VDD is also particularly common among women with PCOS. Estimates range from 40% to 80%, and deficiency is linked to adverse metabolic and reproductive outcomes [3, 10–12]. While some studies suggest that VD supplementation could improve metabolic or reproductive factors, its effect on mood has not been adequately investigated [13–16].

Studies have found that women with PCOS tend to experience lower quality of life and higher levels of DSs [17, 18]. In addition to the inflammatory and neuroendocrine aspects of PCOS, diminished VD levels have been associated with mood disturbances in perinatal populations [19].

The aim of the study was to determine the relation between VD levels and Beck Depression Inventory (BDI) scores in women with PCOS.

Methods

Study design and participants

The study was prepared in accordance with the Declaration of Helsinki and was approved by the Çanakkale Onsekiz Mart University, Faculty of Medicine, Clinical Research Ethics Committee, Çanakkale, Türkiye (No. 2023/11-02, from August 16, 2023). All participants provided written informed consent after receiving a comprehensive explanation of the study's aims, procedures, potential risks, and confidentiality safeguards. No incentives were contingent on questionnaire responses.

This study was designed as a single-center, analytical cross-sectional study conducted exclusively among women diagnosed with PCOS. The primary analytical framework involved stratifying participants according to VD status (deficient vs. non-deficient) to examine its association with DS severity. No external non-PCOS control group was included in the primary statistical analyses. The study included 80 consecutive women, aged 18–45 years, who had received a clinical diagnosis of PCOS according to contemporary, guideline-consistent criteria at the time of recruitment [2, 20]. The diagnosis of PCOS was determined by a specialist physician using clinical or biochemical hyperandrogenism and ultrasonographic evaluation according to the 2003 Rotterdam criteria [21].

Inclusion criteria were reproductive age, a confirmed PCOS diagnosis, and the ability to complete self-report measures in Turkish. Exclusion criteria included current pregnancy or being less than 6 months postpartum, a major known psychiatric disorder other than unipolar depression, endocrine disorders that mimic PCOS (e.g., nonclassic congenital adrenal hyperplasia, Cushing syndrome, hyperprolactinemia, and thyroid dysfunction not on stable therapy), chronic kidney or liver disease, an active infection or inflammatory disease flare-up within the last 4 weeks, high-dose VD therapy (> 2,000 IU/day within the last 3 months) or recent parenteral VD supplementation, and photosensitizing drugs that could alter VD status. Current use of antidepressants or anxiolytics was recorded and considered in sensitivity analyses.

The following parameters were recorded: age, parity, smoking status, physical activity (self-reported categories), and prior psychiatric diagnosis. Anthropometric measurements included height, weight, and body mass index (BMI). Fasting glucose and insulin were measured to compute the homeostasis model assessment of insulin resistance (HOMA-IR) [22], where available. In addition, self-reported information on prior psychiatric history and current use of psychopharmacological medications (including antidepressants and anxiolytics) was collected. Physical activity was assessed using categorical self-report measures. Although the season of blood sampling was recorded as a proxy for sunlight exposure, detailed quantitative measures such as daily sunlight exposure duration, dietary VD intake, and over-the-counter supplementation were not systematically assessed.

BDI is a 21-item self-report scale that assesses the current severity of DSs. Each item is rated on a 4-point scale (0–3), with possible total scores ranging from 0 to 63. A cutoff score of 17 or higher indicates depression. The Turkish validity and reliability of the scale were established by Hisli [23]. BDI scores of 11 or higher were considered indicative of depression: scores 11–16 indicate mild DSs, and scores of 17 or higher indicate moderate to severe DSs [12, 23, 24]. Standard severity ranges were used for descriptive reporting, while analyses modeled scores continuously.

Serum 25(OH)D levels were measured in fasting blood samples using a standardized method aligned with the Vitamin D Standardization Program, where available [25, 26]. The institutional laboratory documented internal quality controls and interassay coefficients of variation. Additional routine analytes (e.g., thyroid-stimulating hormone – TSH) were assessed as required for clinical purposes. Reference ranges for routine biochemical parameters were determined according to the institutional laboratory standards and current international clinical guidelines.

The primary exposure was defined as VDD, *a priori* defined as 25(OH)D < 20 ng/mL (50 nmol/L). This threshold is widely used in clinical and research contexts, particularly before the Endocrine Society shifted away from fixed sufficiency cut-points in 2024. To ensure comparability, we present our findings using this conventional threshold and provide sensitivity analyses using alternative cutoffs (below 12 ng/mL and between 12 and 20 ng/mL). These alternative cutoffs reflect updated perspectives distinguishing VDD from insufficiency [25, 27]. The VD-deficient group (Group 1) included 40 patients with serum 25(OH)D levels below

20 ng/mL. VD-sufficient group (Group 2) included 40 women with serum 25(OH)D levels at or above 20 ng/mL.

Sample size and power

A *post hoc* precision assessment indicated that, with n = 80 and an anticipated standard deviation (SD) of ~ 9–10 points for the BDI, the study could detect a between-group difference of ~ 5 points with 80% power at $\alpha = 0.05$ (two-tailed), or a standardized effect size of ~ 0.5. These estimates are typical for mood symptom studies in PCOS and inform the interpretation of null vs. positive findings in sensitivity checks.

Statistical analysis

Descriptive statistics were used to summarize the characteristics of the sample. Group comparisons by VD status used *t*-tests, Mann-Whitney *U* tests, or Chi-squared/Fisher's exact tests as appropriate. The primary analysis used multivariable linear regression with the BDI score as the dependent variable and VDD as the main predictor. The analysis was adjusted for the prespecified confounders of age, BMI, season (winter/spring vs. summer/autumn), and HOMA-IR (or metformin use when HOMA-IR was unavailable). Model diagnostics were used to evaluate linearity, residual normality, collinearity (variance inflation factors – VIF < 5), and influential observations. Sensitivity analyses included excluding participants taking antidepressants, performing ordinal regression across BDI severity strata, and evaluating alternative VD thresholds. Two-sided $p < 0.05$ denoted statistical significance. The statistical analyses were performed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). All analyses were restricted to the PCOS cohort, and comparisons were performed between VD status subgroups rather than between PCOS and non-PCOS populations. Sensitivity analyses were conducted to evaluate the robustness of findings after excluding participants using antidepressant or anxiolytic medications.

Variables included in the study were selected based on *a priori* clinical relevance and evidence from a multivariable logistic regression model in the literature indicating their potential associations with both VD status and DSs. Specifically, age, BMI, and insulin resistance (HOMA-IR) were included as key confounders. Given the relatively modest sample size, the number of covariates was restricted to minimize the risk of model overfitting. Multicollinearity was assessed using VIF, and model assumptions were evaluated prior to final model specification.

The discriminative performance of the logistic regression model was further evaluated using receiver operating characteristic (ROC) curve analysis, and the area under the curve (AUC) was calculated.

Results

Participants with PCOS were stratified according to serum 25(OH)D status into two groups: Group 1 (n = 40) with serum 25(OH)D < 20 ng/mL and Group 2 (n = 40) with serum 25(OH)D ≥ 20 ng/mL (Table 1). The average age of the

Table 1

Sociodemographic and lifestyle characteristics of women with PCOS according to 25(OH)D level

Variables	Group 1 (n = 40)	Group 2 (n = 40)	*p*-value
Age, years	25.08 ± 0.80	25.80 ± 1.04	0.58
Education level			0.09
elementary	2 (2.5)	8 (10.0)	
high school	14 (17.5)	14 (17.5)	
university and higher	24 (30.0)	18 (22.5)	
Employment status			0.78
employed	16 (20.0)	14 (17.5)	
student	1 (1.3)	2 (2.5)	
unemployed	23 (28.7)	24 (30.0)	
Place of residence			0.49
urban	25 (31.3)	21 (26.3)	
rural	15 (18.7)	19 (23.7)	
Smoking status			0.43
never	28 (35.0)	32 (40.0)	
former	12 (15.0)	8 (10.0)	
Physical activity			0.79
yes	10 (12.5)	9 (11.3)	
no	30 (37.5)	31 (38.7)	
Family history of PCOS			0.80
yes	12 (15.0)	13 (16.3)	
no	28 (35.0)	27 (33.7)	

PCOS – polycystic ovary syndrome; 25(OH)D – 25-hydroxyvitamin D; n – number of respondents.
Data are presented as mean ± standard deviation and as numbers (percentages).
***Note*: Group 1 consisted of respondents with 25(OH)D < 20 ng/mL, while Group 2 consisted of respondents with 25(OH)D ≥ 20 ng/mL.**

patients in Group 1 was 25.08 ± 0.80 years, while the average age of the patients in Group 2 was 25.80 ± 1.04 years (p = 0.58). No statistically significant differences were observed in educational status, employment, place of residence, smoking status, physical activity, or family history of PCOS between the groups (p > 0.05). However, participants in Group 1 had significantly higher BDI scores than those in Group 2 (p = 0.04). The mean difference in BDI scores between the groups was approximately 4.4 points. Additionally, participants with VDD exhibited higher DS severity than those without deficiency (p = 0.02) (Table 2).

Correlation analysis performed across the entire sample demonstrated a significant inverse relationship between serum 25-(OH) D levels and BDI scores (r = -0.23, p = 0.04). Lower VD levels were associated with higher DS severity.

A multivariate logistic regression analysis was performed to determine whether VDD was independently associated with DSs (BDI ≥ 11). After adjusting for age, BMI, and HOMA-IR, VDD remained significantly associated with an increased likelihood of DSs [odds ratio (OR) = 2.84, 95% confidence interval (CI): 1.03–7.82, p = 0.04] (Figures 1 and 2). None of the other variables included in the model showed a

Table 2

Clinical and biochemical characteristics according to 25(OH)D level

Variables	Reference ranges	Group 1 (n = 40)	Group 2 (n = 40)	*p*-value
BMI, kg/m²	18.5–22.4	25.46 ± 1.03	26.50 ± 0.90	0.45
Glucose, mg/dL	70–99	89.72 ± 1.76	89.09 ± 2.28	0.82
Total cholesterol, mg/dL	< 200	171.47 ± 5.01	185.78 ± 6.55	0.08
LDL-C, mg/dL	< 100	95.78 ± 4.51	107.80 ± 5.89	0.42
HDL-C, mg/dL	> 50 (women)	55.85 ± 1.51	63.95 ± 2.49	0.03
Triglycerides, mg/dL	< 150	105.74 ± 8.90	108.27 ± 7.37	0.82
HOMA-IR	< 2.5*	2.92 ± 0.24	4.67 ± 1.03	0.10
BDI score	0–10 normal**	15.85 ± 1.84	11.40 ± 1.20	0.04
BDI ≥11		25 (31.25)	14 (17.50)	0.02

25(OH)D – 25-hydroxyvitamin D; n – number of respondents; BMI – body mass index; LDL-C – low-density lipoprotein cholesterol; HDL-C – high-density lipoprotein cholesterol; HOMA-IR – homeostasis model assessment of insulin resistance; BDI – Beck Depression Inventory.

Data are presented as mean ± standard deviation and as numbers (percentages).

***Note*: Group 1 consisted of respondents with 25(OH)D < 20 ng/mL, while Group 2 consisted of respondents with 25(OH)D ≥ 20 ng/mL. *HOMA-IR values < 2.5 were considered within the normal range. **BDI scores: 0–10 minimal/no depressive symptoms; 11–16 mild depressive symptoms; ≥ 17 moderate-to-severe depressive symptoms.**

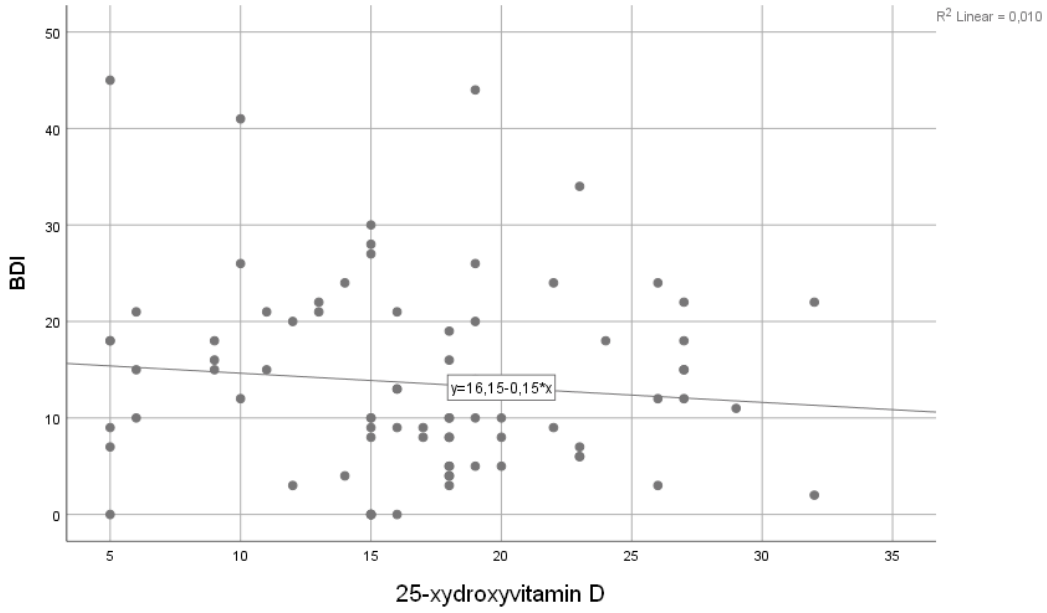

Fig. 1 – Scatter plot illustrating the inverse relationship between serum 25-xydroxyvitamin D levels and Beck Depression Inventory (BDI) scores.

The fitted linear regression line demonstrates a modest negative correlation (r = - 0.23, p = 0.04).

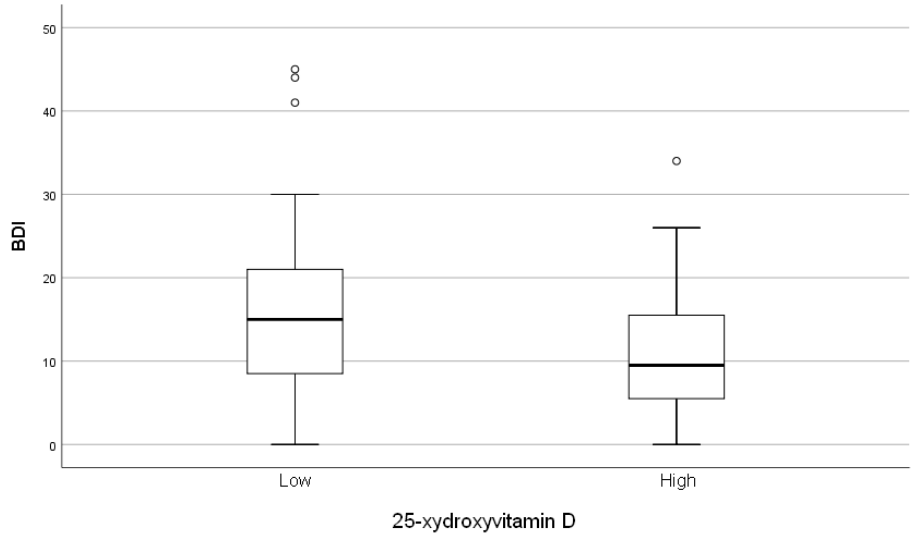

Fig. 2 – Box plot comparing Beck Depression Inventory (BDI) score distributions between participants with 25-xydroxyvitamin D deficiency (< 20 ng/mL) and those with sufficient levels (≥ 20 ng/mL).

Table 3

Multivariate logistic regression analysis for depressive symptoms

Variable	OR	95% CI	*p*-value
25(OH)D	2.84	1.03–7.82	0.04
Age	1.05	0.88–1.25	0.56
BMI	1.08	0.83–0.99	0.32
HOMA-IR	1.06	0.94–1.19	0.32

OR – odds ratio; CI – confidence interval; 25(OH)D – 25-hydroxyvitamin D; BMI – body mass index; HOMA-IR – homeostasis model assessment of insulin resistance.

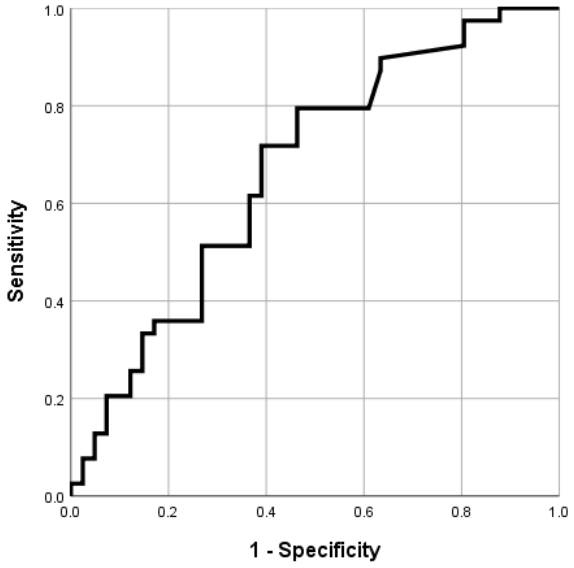

Fig. 3 – Receiver operating characteristic (ROC) curve demonstrating the discriminative performance of the multivariable logistic regression model for predicting depressive symptoms (Beck Depression Inventory – BDI ≥ 11).
***Note*: Diagonal segments are produced by ties.**

statistically significant association with DSs (Table 3). The ROC curve analysis was performed to evaluate the discriminative performance of the multivariable logistic regression model. The model demonstrated acceptable discrimination, with an AUC of 0.668 (95% CI, p = 0.010) (Figure 3).

Discussion

This study examined the association between serum VD levels and DS severity in women with PCOS. Importantly, it is a within-PCOS analysis rather than a case–control comparison. The study found that low VD levels were associated with higher BDI scores in individuals diagnosed with PCOS. This finding aligns with previous studies that have linked low serum 25(OH)D levels to DSs [3, 17, 18]. Our results support studies indicating that women with PCOS experience high levels of depressive symptomatology and compromised quality of life [17, 28]. Furthermore, there is evidence that VDD is prevalent in individuals with PCOS and that correcting it could potentially improve non-mood symptoms [3, 7, 16, 19]. Lifestyle and socioeconomic factors may also confound the observed association. The ROC analysis showed acceptable but limited discriminative performance of the model. However, the modest AUC value suggests that VD status and the included covariates alone may not fully explain the variability in DSs, highlighting the multifactorial nature of depression in women with PCOS.

These findings are biologically plausible *via* converging pathways. First, VD affects serotonin generation by regulating the expression of tryptophan hydroxylase isoforms. This may influence mood regulation [29]. Secondly, VD has immune-modulating functions that reduce cytokine-induced "sickness behavior". This action is increasingly being linked to DSs [8, 30]. Third, PCOS is characterized by low-grade chronic inflammation. This includes elevated levels of C-reactive protein and interleukin-6, as well as changes in immune cell profiles that may increase susceptibility to mood changes when VD levels are low [7, 8, 30]. Furthermore, insulin resistance and excess weight, which are common in PCOS, are associated with lower serum 25(OH)D levels due to mechanisms such as dilution and sequestration, as well as with DSs. These factors present potential feedback loops.

Although the difference in BDI scores between groups was statistically significant, its clinical relevance warrants careful interpretation. The observed mean difference of approximately 4–5 points may reflect a shift within adjacent severity categories (e.g., from minimal to mild or from mild to moderate DSs), rather than a clear threshold for clinical diagnosis or intervention. Therefore, while the findings suggest a greater burden of DSs among women with VDD, they do not necessarily indicate clinically actionable depression requiring treatment. From a clinical perspective, these findings may help identify subgroups of patients with PCOS who are more vulnerable to depressive symptomatology. However, the results should not be interpreted as sufficient to guide clinical decision-making in isolation. Comprehensive psychiatric evaluation and established clinical criteria remain essential for diagnosis and management. Accordingly, the observed association should be considered clinically informative but not practice-changing.

Randomized trials in the general population produce inconsistent results. For instance, the large Vitamin D and Omega-3 Trial-Depression Endpoint Prevention – VITAL-DEP trial did not observe a reduction in new depression cases among adults with adequate VD levels [11]. However, meta-analyses of individuals with VDD or clinical depression indicate small yet significant symptom reductions with supplementation. These findings suggest that baseline VD levels and clinical presentation are important factors [7, 25, 31, 32]. In PCOS, newer meta-analyses indicate that VD supplementation can improve certain metabolic, hormonal, and reproductive indices [10, 11, 16, 19, 25, 27, 32]. However, mood has rarely been the primary focus. Our findings emphasize the need for PCOS-specific randomized controlled trials to address VDD and evaluate mood outcomes.

In clinical practice, the updated 2024 Endocrine Society guidelines advise against screening the general population. The guidelines advocate moving away from very strict sufficiency thresholds but support targeted testing in high-risk individuals or those presenting symptoms [27]. Individuals with PCOS who exhibit symptoms of depression, particularly those related to winter or higher-latitude environments, could fall into this high-risk category. From a clinical perspective, these findings highlight a potential area of interest rather than a basis for intervention. Any consideration of VD assessment or supplementation in this population should be guided by existing clinical guidelines rather than the results of this study alone. Where deficiency has been confirmed, supplementation should adhere to current safety guidelines and consider repletion strategies [10, 27, 30, 33].

The findings of this study indicate a significant association between VDD and increased DS severity among women with PCOS. However, these results should be interpreted as evidence of an association rather than as direct evidence of a therapeutic effect of VD on DSs. Given the cross-sectional nature of the study, causal inferences cannot be established. Nevertheless, several biological mechanisms may help explain the observed relationship.

VD plays an important role in neurobiological processes related to mood regulation. Experimental and clinical evidence suggests that VD may influence serotonin synthesis through the regulation of tryptophan hydroxylase expression, thereby contributing to the modulation of emotional states and depressive symptomatology [29, 30]. In addition, VD has immunomodulatory effects and may reduce systemic inflammation by regulating proinflammatory cytokines. Since chronic low-grade inflammation is frequently observed in PCOS, this pathway may partially explain the association between VDD and DSs. Furthermore, VD has been implicated in the regulation of the hypothalamic-pituitary-adrenal axis, which is closely linked to stress response and mood disorders.

Despite these plausible mechanisms, the cross-sectional design of the present study precludes determining whether VDD contributes to DSs or whether DSs themselves influence lifestyle behaviors that affect VD status, such as reduced outdoor activity or dietary intake. Therefore, the findings should be interpreted cautiously. Longitudinal studies and randomized controlled trials focusing specifically on mood outcomes are required to clarify the direction and clinical significance of this relationship.

While the association between VDD and DSs observed in this study is noteworthy, it should be interpreted with cau-

tion. The findings do not support causal inference, nor do they justify immediate changes in clinical practice. Instead, they should be considered hypothesis-generating and serve as a basis for future prospective and interventional research.

Limitations of the study

The strengths of the current study include same-day assessments of mood and VD levels, predetermined adjustments for confounding factors, and sensitivity analyses based on new guidelines and alternative cutoff points. This study has several limitations that should be considered when interpreting the findings. First, the cross-sectional design precludes the establishment of causal relationships between VDD and DSs. Second, the study was conducted at a single center with a relatively modest sample size, which may limit the generalizability of the findings. Third, although several potential confounders—such as age, BMI, season, and insulin resistance—were adjusted for, other factors that may influence VD levels, including detailed dietary intake, precise sunlight exposure, and duration of outdoor activity, were not quantitatively assessed. In addition, PCOS phenotypes were not analyzed separately due to the limited sample size, which may have influenced the interpretation of the results. Finally, DSs were assessed using a self-report instrument rather than a structured psychiatric interview. Additional multicenter longitudinal studies with larger samples and more comprehensive assessments of lifestyle factors are needed to further clarify the relationship between VD status and DSs in women with PCOS.

Despite adjustment for several key confounders, the possibility of residual confounding cannot be excluded. Important variables known to influence both VD status and DSs—such as quantitative sunlight exposure, dietary VD intake, socioeconomic status, and detailed physical activity patterns—were not comprehensively measured. Although seasonality was included as a proxy for sunlight exposure and self-reported physical activity was recorded, these measures may not fully capture individual variability. In addition, while psychopharmacological medication use and prior psychiatric history were documented and considered in sensitivity analyses, the study was not powered to fully disentangle their potential modifying effects. These limitations should be considered when interpreting the observed associations.

Conclusion

Our findings indicate that lower vitamin D levels are associated with greater depressive symptom severity among women with polycystic ovary syndrome. However, given the cross-sectional design of the study, these results should be interpreted as evidence of association rather than causation. The observed relationship may reflect complex interactions between biological, behavioral, and environmental factors. Therefore, no direct clinical implications regarding vitamin D supplementation or screening can be inferred from the present findings. Future longitudinal studies and randomized controlled trials are required to clarify the directionality and clinical relevance of this association. The clinical significance of these findings remains to be clarified in prospective studies evaluating patient-centered outcomes.

Conflict of interest

The authors declare no conflict of interest.

R E F E R E N C E S

1. *Brutocao C, Zaiem F, Alsawas M, Morrow AS, Murad MH, Javed A.* Psychiatric disorders in women with polycystic ovary syndrome: a systematic review and meta-analysis. Endocrine 2018; 62(2): 318–25. DOI: 10.1007/s12020-018-1692-3.
2. *Teede HJ, Tay CT, Laven JJE, Dokras A, Moran LJ, Piltonen TT,* et al. Recommendations from the 2023 international evidence-based guideline for the assessment and management of polycystic ovary syndrome. J Clin Endocrinol Metab 2023; 108(10): 2447–69. DOI: 10.1210/clinem/dgad463.
3. *Cooney LG, Lee I, Sammel MD, Dokras A.* High prevalence of moderate and severe depressive and anxiety symptoms in polycystic ovary syndrome: a systematic review and meta-analysis. Hum Reprod 2017; 32(5): 1075–91. DOI: 10.1093/humrep/dex044.
4. *Yadav S, Delau O, Bonner AJ, Markovic D, Patterson W, Ottey S,* et al. Direct economic burden of mental health disorders associated with polycystic ovary syndrome: Systematic review and meta-analysis. ELife 2023; 12: e85338. DOI: 10.7554/eLife.85338.
5. *Arayıcı ME, Korkut-Uysal SO, Leblebici A, Akcali Z, Edizer DT, Kabul S,* et al. Evaluation of 25-hydroxyvitamin D (25(OH)D) levels before and during the COVID-19 pandemic: A cross-sectional study and trend analysis involving 86,772 samples. PLoS One 2023; 18(5): e0284647. DOI: 10.1371/journal.pone.0284647.
6. *Płudowski P, Kos-Kudła B, Walczak M, Fal A, Zozulińska-Ziółkiewicz D, Sieroszewski P,* et al. Guidelines for Preventing and Treating Vitamin D Deficiency: A 2023 Update in Poland. Nutrients 2023; 15(3): 695. DOI: 10.3390/nu15030695.
7. *Li L, Yu J, Zhou Z.* Association between neutrophil-to-lymphocyte ratio and polycystic ovary syndrome: A PRISMA-compliant systematic review and meta-analysis. Medicine (Baltimore) 2022; 101(38): e30579. DOI: 10.1097/MD.0000000000030579.
8. *Loftis JM, Huckans M, Morasco BJ.* Neuroimmune mechanisms of cytokine-induced depression: current theories and novel treatment strategies. Neurobiol Dis 2010; 37(3): 519–33. DOI: 10.1016/j.nbd.2009.11.015.
9. *Zhai Y, Pang Y.* Systemic and ovarian inflammation in women with polycystic ovary syndrome. J Reproduc Immunol 2022; 151: 103628. DOI: 10.1016/j.jri.2022.103628.
10. *Lips P, de Jongh RT, van Schoor NM.* Trends in Vitamin D Status Around the World. JBMR Plus 2021; 5(12): e10585. DOI: 10.1002/jbm4.10585.
11. *Okereke OI, Reynolds CF 3rd, Mischoulon D, Chang G, Vyas CM, Cook NR,* et al. Effect of Long-term Vitamin D3 Supplementation vs Placebo on Risk of Depression or Clinically Relevant Depressive Symptoms and on Change in Mood Scores: A Randomized Clinical Trial. JAMA 2020; 324(5): 471–80. DOI: 10.1001/jama.2020.10224.
12. *Wang YP, Gorenstein C.* Psychometric properties of the Beck Depression Inventory-II: a comprehensive review. Braz J Psychiatry 2013; 35(4): 416–31. DOI: 10.1590/1516-4446-2012-1048.
13. *Cochrane KM, Bone JN, Williams BA, Karakochuk CD.* Optimizing vitamin D status in polycystic ovary syndrome: a systematic review and dose-response meta-analysis. Nutr Rev 2024; 82(9): 1176–86. DOI: 10.1093/nutrit/nuad117.

14. *Cui A, Zhang T, Xiao P, Fan Z, Wang H, Zhuang Y.* Global and regional prevalence of vitamin D deficiency in population-based studies from 2000 to 2022: A pooled analysis of 7.9 million participants. Front Nutr 2023; 10: 1070808. DOI: 10.3389/fnut.2023.1070808.
15. *Thiele DK, Senti JL, Anderson CM.* Maternal vitamin D supplementation to meet the needs of the breastfed infant: a systematic review. J Hum Lact 2013; 29(2): 163–70. DOI: 10.1177/0890334413477916.
16. *Wen X, Wang L, Li F, Yu X.* Effects of vitamin D supplementation on metabolic parameters in women with polycystic ovary syndrome: a randomized controlled trial. J Ovarian Res 2024; 17(1): 147. DOI: 10.1186/s13048-024-01473-6.
17. *Kocak DY, Ugurlu M.* Depression symptoms and quality of life in women with polycystic ovary syndrome. Perspect Psychiatr Care 2022; 58(4): 2837–45. DOI: 10.1111/ppc.13131.
18. *Fallah M, Askari G, Asemi Z.* Is Vitamin D Status Associated with Depression, Anxiety and Sleep Quality in Pregnancy: A Systematic Review. Adv Biomed Res 2020; 9: 32. DOI: 10.4103/abr.abr_188_19.
19. *Sağlam Akkaya G, Pektaş G, Karakullukcu S, Pektaş A, Sağlam Aykut D.* Vitamin D Deficiency is Associated with Depression, Anxiety and Sleep Disturbance in Pregnant Women. J Acad Res Med 2021; 11(1): 51–5. DOI: 10.4274/jarem.galenos.2021.3897.
20. *Forslund M, Melin J, Stener-Victorin E, Hirschberg AL, Teede H, Vanky E,* et al. International evidence-based guideline on assessment and management of PCOS—A Nordic perspective. Acta Obstet Gynecol Scand 2024; 103(1): 7–12. DOI: 10.1111/aogs.14725.
21. *Rotterdam ESHRE/ASRM-Sponsored PCOS consensus workshop group.* Revised 2003 consensus on diagnostic criteria and long-term health risks related to polycystic ovary syndrome (PCOS). Hum Reprod 2004; 19(1): 41–7. DOI: 10.1093/humrep/deh098.
22. *Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC.* Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 1985; 28(7): 412–9. DOI: 10.1007/BF00280883.
23. *Hisli NS.* Use of the Beck Depression Inventory with Turkish University Students: Reliability, validity and Factor Analysis. Türk Psikoloji Dergisi 1989.
24. *Uslu RI, Kapci EG, Oncu B, Ugurlu M, Turkcapar H.* Psychometric properties and cut-off scores of the Beck Depression Inventory-II in Turkish adolescents. J Clin Psychol Med Settings 2008; 15(3): 225–33. DOI: 10.1007/s10880-008-9122-y.
25. *Grant WB, Wimalawansa SJ, Pludowski P, Cheng RZ.* Vitamin D: Evidence-Based Health Benefits and Recommendations for Population Guidelines. Nutrients 2025; 17(2): 277. DOI: 10.3390/nu17020277.
26. *Kazeminia M, Rajati F, Rasulehvandi R, Rajati M.* The effect of vitamin D on the hormonal profile of women with polycystic ovarian syndrome: a systematic review and meta-analysis. Middle East Fertil Soc J 2024; 29(1): 45. DOI: 10.1186/s43043-024-00201-w.
27. *Demay MB, Pittas AG, Bikle DD, Diab DL, Kiely ME, Lazaretti-Castro M,* et al. Vitamin D for the Prevention of Disease: An Endocrine Society Clinical Practice Guideline. J Clin Endocrinol Metab 2024; 109(8): 1907–47. DOI: 10.1210/clinem/dgae290. Erratum in: J Clin Endocrinol Metab 2025; 110(3): e916. DOI: 10.1210/clinem/dgae854. Erratum in: J Clin Endocrinol Metab 2025; 110(8): e2810. DOI: 10.1210/clinem/dgaf310.
28. *Escobar-Morreale HF.* Polycystic ovary syndrome: definition, aetiology, diagnosis and treatment. Nat Rev Endocrinol 2018; 14(5): 270–84. DOI: 10.1038/nrendo.2018.24
29. *Patrick RP, Ames BN.* Vitamin D hormone regulates serotonin synthesis. Part 1: relevance for autism. FASEB 2014; 28(6): 2398–413. DOI: 10.1096/fj.13-246546.
30. *Nusslock R, Alloy LB, Brody GH, Miller GE.* Annual Research Review: Neuroimmune network model of depression: a developmental perspective. J Child Psychol Psychiatry 2024; 65(4): 538–67. DOI: 10.1111/jcpp.13961.
31. *Ghaemi S, Zeraattalab-Motlagh S, Jayedi A, Shab-Bidar S.* The effect of vitamin D supplementation on depression: a systematic review and dose-response meta-analysis of randomized controlled trials. Psychol Med 2024; 54(15): 3999–4008. DOI: 10.1017/S0033291724001697.
32. *Yang M, Shen X, Lu D, Peng J, Zhou S, Xu L,* et al. Effects of vitamin D supplementation on ovulation and pregnancy in women with polycystic ovary syndrome: a systematic review and meta-analysis. Front Endocrinol (Lausanne) 2023; 14: 1148556. DOI: 10.3389/fendo.2023.1148556.
33. *Osredkar J, Vičič V, Hribar M, Benedik E, Siuka D, Jerin A,* et al. Seasonal variation of total and bioavailable 25-hydroxyvitamin D [25(OH)D] in the healthy adult Slovenian population. Acta Biochem Pol 2024; 71: 13108. DOI: 10.3389/abp.2024.13108.

Received on September 25, 2025
Revised on March 7, 2026
Revised on April 26, 2026
Accepted on May 3, 2026
Online First June 2026

SHORT REPORT

UDC: 616.28-008.14-089-053.2:534.773
DOI: https://doi.org/10.2298/VSP251225037B

BAHA® implantation in pediatric patients: indications and clinical outcomes

Ugradnja aparata BAHA® bolesnicima dečjeg uzrasta: indikacije i rezultati

Ivan Baljošević*†, Ljiljana Čvorović‡§, Maša Petrović‡‖, Svetozar Memarović§

***Institute for Mother and Child Health Care of Serbia "Dr. Vukan Čupić", Department of Pediatric Otorhinolaryngology, Belgrade, Serbia; †University of Business Academy, Faculty of Dentistry, Pančevo, Serbia; ‡University of Belgrade, Faculty of Medicine, Belgrade, Serbia; §University Clinical Center of Serbia, Clinic for Otorhinolaryngology and Maxillofacial Surgery, Belgrade, Serbia; ‖Institute for Cardiovascular Diseases "Dedinje", Belgrade, Serbia**

Abstract

Background/Aim. Bone-anchored hearing aids (BAHA®) are implantable devices that transmit sound through bone conduction directly to the inner ear. They are indicated for patients with conductive or mixed hearing loss who cannot benefit from conventional hearing aids. The aim of this study was to evaluate surgical outcomes, postoperative complications, and audiological improvements following percutaneous and transcutaneous BAHA® implantation in a pediatric cohort. **Methods.** A retrospective cohort study was carried out on 20 children who underwent BAHA® implantation between 2011 and 2024. Demographic data, clinical indications, syndromic associations, audiological findings, and postoperative complications were analyzed. Statistical analyses included Fisher's exact test and the Wilcoxon signed-rank test, with significance set at $p < 0.05$. **Results.** Out of the total of 20 patients (60% male; mean age 6.3 years), 11 received percutaneous and 9 transcutaneous BAHA® devices. The primary indications were congenital anomalies (50%), notably Treacher-Collins syndrome and isolated atresia. In patients with a percutaneous BAHA® device, skin infections were recorded in 3 (27%) of 11 patients, and they were accompanied by skin overgrowth, which required revision surgery in 2 (18%) patients. In the group implanted with the transcutaneous BAHA® Attract device, paresthesia and skin erythema were observed in one case each. The mean hearing improvement was 36.6 dB with percutaneous BAHA® devices and 31.7 dB with BAHA® Attract devices at 0.5 kHz, 1 kHz, 2 kHz, and 4 kHz. Significant postoperative hearing improvement was achieved in all patients ($p = 0.0002$). **Conclusion.** BAHA® implantation provides significant hearing rehabilitation in children with conductive or mixed hearing loss. The transcutaneous system demonstrates comparable audiological outcomes with fewer complications and superior cosmetic acceptability compared to the percutaneous system.

Keywords:
child; child, preschool; hearing aids; hearing loss, conductive; hearing loss, sensorineural; otorhinolaryngologic surgical procedures; treatment outcome.

Apstrakt

Uvod/Cilj. *Bone-anchored hearing aids* (BAHA®) su implantabilna pomagala kojima se, putem koštane provodljivosti, zvuk prenosi direktno u unutrašnje uvo. Indikovani su za obolele od konduktivnog ili mešovitog gubitka sluha, koji ne mogu imati koristi od konvencionalnih slušnih pomagala. Cilj rada bio je da se procene hirurški ishodi, postoperativne komplikacije i audiološka poboljšanja posle perkutane i transkutane implantacije BAHA® u pedijatrijskoj kohorti. **Metode.** Retrospektivna kohortna studija sprovedena je na 20-oro dece, kojima je izvršena ugradnja uređaja BAHA® između 2011. i 2024. godine. Analizirani su demografski podaci, kliničke indikacije, udruženost sa sindromima, audiološki nalazi i postoperativne komplikacije. Statistička analiza uključila je Fisher-ov egzaktni test i Wilcoxon-ov test uparenih rangova, pri čemu je nivo statističke značajnosti postavljen na $p < 0,05$. **Rezultati.** Od ukupno 20 bolesnika (60% muškog pola; prosečanog uzrasta 6,3 godine), kod 11 je ugrađen perkutani, a kod 9 transkutani aparat BAHA®. Primarne indikacije bile su kongenitalne anomalije (50%), naročito Treacher-Collins-ov sindrom i izolovana atrezija. Kod bolesnika sa perkutanim BAHA® aparatom registrovane su infekcije kože kod 3 (27%) od 11 bolesnika i bile su praćene hipertrofijom kože koje su zahtevale reintervenciju kod dvoje (18%) dece. U grupi kod

Correspondence to: Ivan Baljošević, Mother and Child Health Institute of Serbia "Dr. Vukan Čupić", Department of Otorhinolaryngology, Radoja Dakića 6, 11 070 Belgrade, Serbia. E-mail: ivan.baljosevic@gmail.com

koje je bio implantiran transkutani BAHA® Attract aparat zabeleženi su parestezija i eritem kože u po jednom slučaju. Prosečno poboljšanje sluha bilo je 36,6 dB sa perkutanim aparatom BAHA®, a 31,7 dB sa aparatom BAHA® Attract na 0.5 kHz, 1 kHz, 2 kHz i 4 kHz. Kod svih bolesnika postignuto je značajno postoperativno poboljšanje sluha ($p = 0{,}0002$). **Zaključak.** Ugradnja aparata BAHA® obezbeđuje značajnu rehabilitaciju sluha kod dece obolele od konduktivnog ili mešovitog oštećenja sluha. Transkutani sistem pokazuje uporedive audiološke ishode uz manje komplikacija i bolju estetsku prihvatljivost u poređenju sa perkutanim sistemom.

Ključne reči:
deca; deca, predškolska; sluh, pomagala; sluh, konduktivni gubitak; sluh, senzornonervni gubitak; hirurgija, otorinolaringološka, procedure; lečenje, ishod.

Introduction

Bone-anchored hearing aids (BAHA®) are specialized hearing devices developed to facilitate sound conduction through bone. This technology, initially introduced in Gothenburg, Sweden, in 1977, uses titanium implants that integrate with bone and are surgically placed behind the ear. BAHA® devices were designed primarily to assist patients with conductive and mixed types of hearing loss by transmitting sound vibrations directly through the bone to the inner ear, bypassing the outer and middle ears [1, 2]. Today, more than 150,000 individuals with hearing impairments use BAHA® devices. These devices are particularly beneficial for patients who are unable to achieve satisfactory hearing improvement with conventional hearing aids, including those with unilateral deafness or conductive/mixed hearing loss.

Two main types of bone conduction implants are now available: percutaneous (transdermal) and transcutaneous (non-penetrating). The percutaneous BAHA® device includes a titanium implant connected to an abutment and sound processor, allowing for direct sound transmission. However, ongoing maintenance and meticulous hygiene are required to prevent complications. The transcutaneous BAHA® Attract system, introduced in 2013, consists of a titanium implant and two magnets: one subcutaneous, connected to the implant, and one external magnet connected to the sound processor. A soft material pad distributes pressure evenly across the skin, improving comfort and reducing the risk of irritation [3]. In cases of insufficient bone thickness, a new opening is created in the bone to allow secure placement of the titanium implant.

This study aimed to assess the surgical outcomes of percutaneous and transcutaneous BAHA® implants, evaluate postoperative complications, and measure improvements in hearing thresholds in pediatric patients who underwent BAHA® implantation at a tertiary care center.

Methods

This retrospective cohort study was conducted at the Institute for Mother and Child Health Care of Serbia "Dr. Vukan Čupić", a tertiary care institution in Belgrade, Serbia. Between 2011 and 2024, BAHA® implants were placed in 20 pediatric patients with conductive or mixed hearing loss. This study was approved by the Ethics Committee of the Institute (Approval No. 8/135, from March 27, 2024).

The study included patients with severe conductive hearing loss due to congenital anomalies or those who had previously undergone surgery for chronic suppurative otitis media with cholesteatoma. Patients with acute unilateral deafness were also included. All of these patients were unable to use conventional hearing aids for auditory rehabilitation due to ear deformities, repeated surgical interventions, or complete deafness. Due to financial constraints, no surgeries were performed from 2012 to 2014, and because of the COVID-19 pandemic, surgical procedures were paused between March 2020 and March 2022. Therefore, this study spans an effective period of 9 years.

The percutaneous BAHA® system was the only available type for implantation and was used until 2017 (Figures 1 and 2). Implantation of the transcutaneous BAHA® Attract system commenced in 2017 (Figures 3 and 4). The

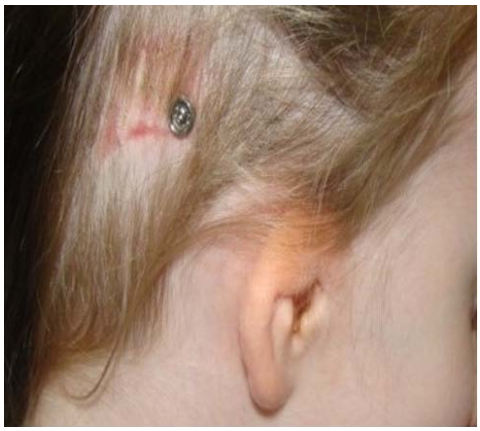

Fig. 1 – Percutaneous BAHA® implant – a titanium implant inserted into the cranial bone *via* a skin incision, with an abutment affixed to it. BAHA – bone-anchored hearing aid.

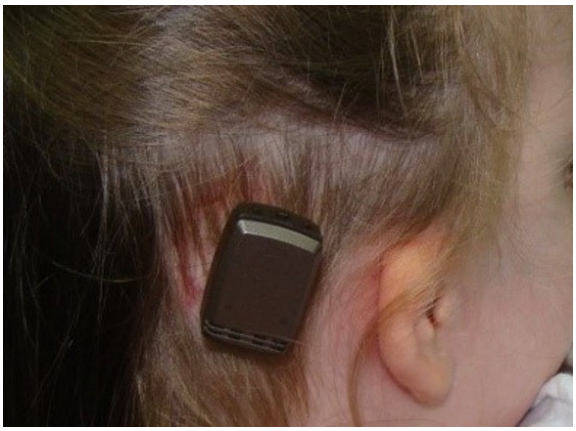

Fig. 2 – BAHA® sound processor attached to the percutaneous abutment. BAHA – bone-anchored hearing aid.

parents were fully informed about the treatment options, risks, and benefits of the procedure, and written consent was obtained prior to surgery.

The baseline data collected included patient demographics, clinical presentation, audiological findings, and any relevant syndromic associations. Preoperative pure-tone audiograms were obtained. In pure-tone audiometry, the hearing threshold for air conduction was calculated as the mean of the thresholds measured at 0.5 kHz, 1 kHz, 2 kHz, and 4 kHz. This threshold was measured in decibels (dB) hearing level (HL). All patients wore a soft-band BAHA® for a period of at least 1 to 6 months before surgery. Patients with conductive hearing loss received BP110 processors, while those with sensorineural hearing loss received BP110P processors. Following the introduction of the BAHA® Attract system (after 2017), the BAHA® 5 Power and BAHA® 6 Max processors were used; these devices were more compact and enabled synchronization with a mobile phone application. Comprehensive pediatric evaluations were conducted to identify other congenital anomalies or syndromic associations. Cochlear™ BAHA® devices (Cochlear, Sweden) were used in all cases.

Surgical procedures for both percutaneous and transcutaneous BAHA® devices followed the manufacturer's guidelines.

Descriptive and analytical statistics were computed using IBM SPSS v21. Descriptive statistics included absolute and relative frequencies (numbers and percentages), measures of central tendency (means), and dispersion (ranges). Analytical tests included Fisher's exact test and the Wilcoxon signed-rank test, with statistical significance set at $p < 0.05$.

Results

General characteristics

The average age of patients was 6.3 years (range 4.5–15 years), with a predominance of males (60%).

The most common indications for implantation included congenital anomalies of the outer and middle ear (50%), consisting of Treacher-Collins syndrome (5 patients), isolated atresia (4 patients), and Crouzon syndrome (1 patient). Other indications were chronic middle ear cholesteatoma (6 patients - 30%), and unilateral deafness in 4 (20%) children (Table 1).

Hearing aid implantation stages

The implantation sites were marked preoperatively, positioning the processor approximately 5–7 cm behind the ear canal at the level of the temporal line. Among 20 patients, 11 (55%) received a percutaneous BAHA® device, while 9 (45%) received a BAHA® Attract device.

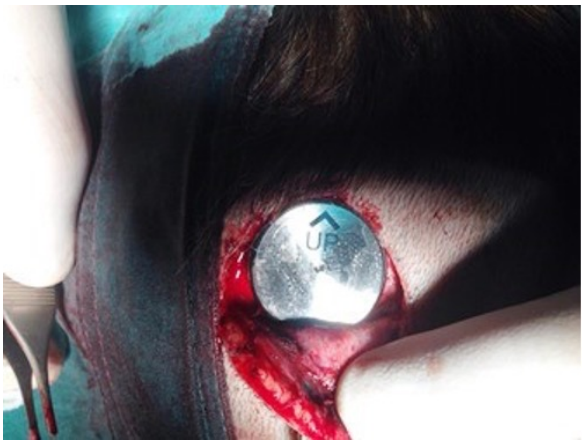

Fig. 3 – BAHA® Attract – magnetic plate positioned on the titanium implant with the "UP" mark facing upward. BAHA – bone-anchored hearing aids.

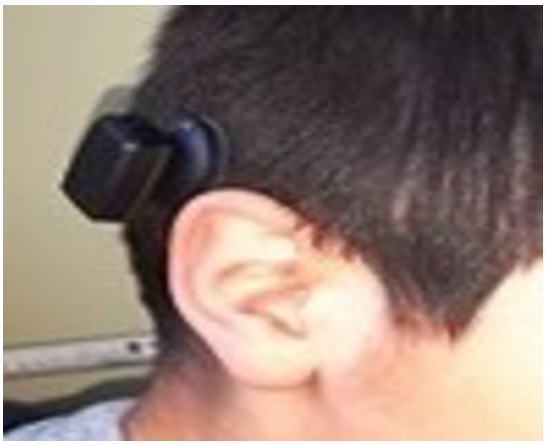

Fig. 4 – BAHA® Attract processor placed over intact skin and hair, directly above the subcutaneous magnetic plate. BAHA – bone-anchored hearing aids.

Table 1

Overview of indications for patients with BAHA® implants

Indication	Percutaneous	Transcutaneous	Total
Congenital anomalies of the outer and middle ear			10 (50)
isolated atresia	1 (25)	3 (75)	4 (20)
Treacher-Collins syndrome	4 (80)	1 (20)	5 (25)
Crouzon syndrome	0 (0)	1 (100)	1 (5)
Chronic suppurative otitis media with cholesteatoma	5 (83.3)	1 (16.7)	6 (30)
Unilateral total deafness	1 (25)	3 (75)	4 (20)

BAHA – bone-anchored hearing aids.
All values are presented as numbers (percentages).
***Note:* Rarer indications are also given as numbers (percentages), but in relation to the three most common indications.**

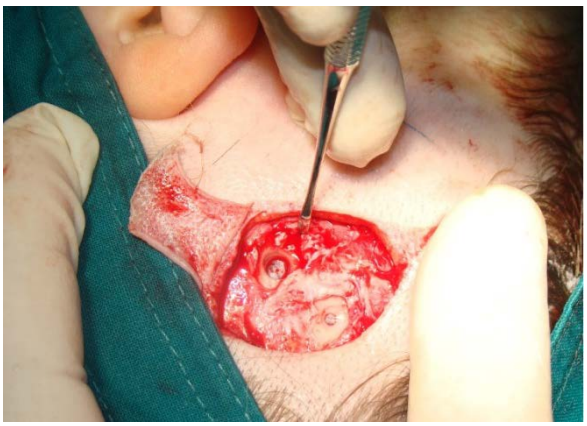

Fig. 5 – New opening created in the direction of the instrument due to insufficient cranial bone thickness to accommodate the titanium implant.

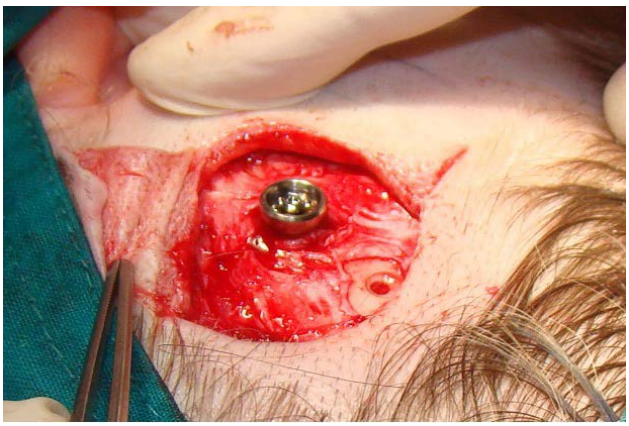

Fig. 6 – Titanium implant and abutment positioned in the newly created, adequately deep opening in the cranial bone.

Soft tissue thickness was less than 6 mm in all cases, allowing for implant placement without soft tissue reduction. A 4-mm implant was used in each case.

During surgery, 14 (70%) patients experienced no complications. In 2 (10%) patients, significant bleeding from the bone was observed, and in 4 (20%) patients, insufficient bone thickness necessitated creating a new opening to ensure stable implant placement (Figures 5 and 6). In one patient, three attempts were required before an adequate bone-thickness site was identified.

The average operative time for percutaneous and transcutaneous BAHA® implantation was similar, ranging from 40 to 47 min, with a mean of 42 min.

Outcomes

Wound healing was uneventful in all cases. The sound processor was connected one month postoperatively.

Preoperative hearing thresholds were calculated as the average of the thresholds measured at 0.5 kHz, 1 kHz, 2 kHz, and 4 kHz. These baseline pure-tone averages were 40–60 dB HL in 4 patients and 60–80 dB HL in 12 patients. The remaining 4 patients presented with complete deafness. Postoperatively, hearing thresholds improved in all patients, reaching 20–40 dB HL across the cohort. The mean hearing improvement was 36.6 dB for percutaneous devices and 31.7 dB for BAHA® Attract at 0.5 kHz, 1 kHz, 2 kHz, and 4 kHz, with an average final threshold of 27 dB. A statistically significant improvement in hearing thresholds was observed postoperatively for both device types ($p = 0.0002$).

In patients with a percutaneous BAHA® device, skin infections were recorded in 3 (27%) out of 11 patients, accompanied by skin overgrowth, which required revision surgery in 2 (18%) patients. In the BAHA® Attract group, paresthesia occurred in one patient and erythema occurred in another; adjustment of the magnet strength resolved these symptoms.

There was no significant difference in complication rates between the percutaneous and transcutaneous BAHA® devices ($p = 0.635$). Although the percutaneous group showed slightly more complications, the difference was not statistically significant. Given the small sample size, the study may be underpowered to detect minor differences in complication rates.

Discussion

The BAHA® device functions by bypassing the hearing mechanism of the outer and middle ear, transmitting sound directly to the cochlea through the cranial bone. Consequently, it is particularly suitable for patients with outer ear deformities, such as microtia and anotia. Patients with chronic suppurative otitis media, adhesive otitis media, external otitis, or frequent outer ear infections who cannot use conventional hearing aids also benefit greatly from BAHA® use [4].

In our study, the most common indication for intervention was congenital anomalies of the outer and middle ear, accounting for 10 (50%) patients, most often presenting as part of various syndromes. Due to these anatomical variations, these patients were unable to use conventional hearing amplifiers, making BAHA® implantation the only viable solution. Additionally, children who have undergone surgery for middle ear cholesteatoma may still experience occasional purulent ear infections, hindering the use of standard hearing aids. Our study included 6 (30%) of such patients.

The indications should have been expanded to include patients with conductive hearing loss, mixed hearing loss, or single-sided deafness. In such cases, sound is transmitted transcranially to the other healthy ear, providing patients with stereo hearing. In our study, 4 (20%) patients fell into the category of unilateral total deafness. The use of BAHA® improves audibility and sound quality, thereby enhancing the overall quality of life for these patients.

Percutaneous BAHA® devices facilitate direct sound transmission but present challenges, including aesthetic concerns and the risk of skin complications at the implant site. In contrast, BAHA® Attract devices offer a transcutaneous solution that minimizes skin complications by shielding the implant from direct contact. This approach eliminates the need for daily skin care at the implant site and provides superior aesthetic outcomes [5, 6].

In our study, the average operative time for percutaneous and transcutaneous BAHA® implantations was similar, ranging from 40 to 47 min, with a mean of 42 min. This aligns with previously reported times in the literature, which range from 37 min to 57 min [7, 8]. During BAHA® Attract implantation, soft tissue reduction may be required, occurring in 11.1% to 31.2% of cases [8, 9], depending on individual variations in skin and subcutaneous tissue thickness. In our cohort, soft tissue reduction was not necessary.

Gawecki et al. [3] reported intraoperative complications in 12.1% of 125 patients receiving BAHA® implants, including bleeding, abnormal bone morphology, and insufficient bone thickness. Hougaard et al. [7] described the use of 3-mm implants in cases where the dura was exposed after drilling to a depth of 3 mm. Major postoperative complications were rare, and healing was uncomplicated in most cases. Minor hematomas were noted in 7.2% of patients on the day following surgery [3]. In our study, intraoperative complications included significant bleeding in 2 patients, which was successfully controlled with wax tamponade. In addition, insufficient bone thickness in 4 children necessitated the creation of a new opening for implant placement.

It is generally accepted that hearing gain with the BAHA® Attract system is lower than with percutaneous BAHA®. Audiometric measurements are performed at 4 and 6 weeks and at 3 and 6 months postoperatively. A gradual improvement in hearing performance up to 3 months, as described by Briggs et al. [10], is established but not statistically significant between the two types of implants. The improvement in hearing performance, particularly between 4 and 6 weeks, is attributed to gradual patient adaptation, progressively improved magnet-to-bone contact, and fine-tuning by the audiologist, with sound amplification gradually increased. After 3 months, a relative stabilization of audiometric threshold levels occurs [11]. Vaughan-Christensen et al. [12] also stated that patients are satisfied with the sound provided by the BAHA® Attract system. They noted that in more recent comparisons, they found no statistically significant group differences between the percutaneous and transcutaneous systems. Iseri et al. [8] also reported similar audiometric thresholds at 0.5 kHz, 1 kHz, 2 kHz, and 4 kHz in adults between the percutaneous and transcutaneous systems.

In our study, the mean hearing improvement was 36.6 dB with percutaneous BAHA® devices and 31.7 dB with BAHA® Attract devices at 0.5 kHz, 1 kHz, 2 kHz, and 4 kHz, with an average aided hearing threshold of 27 dB. Studies by Baker et al. [13] and Powell et al. [14] predominantly investigated pediatric populations, reporting greater improvements in mean aided thresholds (41 dB HL and 30.6 dB HL, respectively) compared with studies involving predominantly adult populations, such as that by Briggs et al. [10], who reported an improvement of 18.4 dB HL. Late complications, defined as those occurring 7 or more days after surgery, are described in the literature. These complications are more frequent and more severe in patients with percutaneous BAHA® devices. In these patients, daily cleaning and maintenance of the skin-penetrating implant site are required, which may lead to skin infections accompanied by excessive skin overgrowth. Thus, poor and irregular hygiene of the skin around the abutment leads to skin hypertrophy, which can even completely cover the implant. In our study, skin infections occurred in 27% of patients with percutaneous implants; skin infection rates reported in the literature range from 8% to 59%. Skin hypertrophy can result in skin overgrowth covering the implant, necessitating revision surgery in 5–42% of cases [15–18]; in our cohort, revision surgery was required in 18% of patients.

In contrast, complications following BAHA® Attract implantation are significantly milder and less frequent. Seroma or hematoma formation has been reported in 4.4% of cases and was managed conservatively [8], while 8.9% of patients experienced numbness around the flap area [9]. In our pediatric cohort with BAHA® Attract system implantation, paresthesia and skin erythema were observed in one case each. In both instances, a reduction of the magnet strength resulted in clinical improvement.

Conclusion

BAHA® implantation has proven to be an effective method of auditory rehabilitation in children with conductive or mixed hearing loss who are unresponsive to conventional hearing aids. This procedure enhances a child's spatial orientation, speech development, and intellectual growth. The BAHA® Attract system, in particular, offers a safe and minimally invasive alternative with rapid healing, excellent aesthetic outcomes, and a reduced risk of complications.

Conflicts of interest

The authors of this paper certify that they have no affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

REFERENCES

1. *Reinfeldt S, Håkansson B, Taghavi H, Eeg-Olofsson M.* New developments in bone-conduction hearing implants: a review. Med Devices (Auckl) 2015; 8: 79–93. DOI: 10.2147/MDER.S39691.
2. *Dimitriadis PA, Farr MR, Allam A and Ray J.* Three-year experience with the cochlear BAHA attracts implant: a systematic review of the literature. BMC Ear Nose Throat Disord 2016; 16: 12. DOI: 10.1186/s12901-016-0033-5.
3. *Gawecki W, Balcerowiak A, Kalinowicz E, Wróbel M.* Evaluation of surgery and surgical results of Baha® Attract system implantations - single centre experience of hundred twenty five

cases. Braz J Otorhinolaryngol 2019; 85(5): 597–602. DOI: 10.1016/j.bjorl.2018.04.011.
4. *Marfatia H, Shah K, Shelke S, Krishnan K.* Baha Attract: Our Experience. Indian J Otolaryngol Head Neck Surg 2017; 69(4): 544–8. DOI: 10.1007/s12070-017-1192-4.
5. *Doshi J, Sheehan P, McDermott AL.* Bone anchored hearing aids in children: an update. Int J Pediatric Otorhinolaryngol 2012; 76(5): 618–22. DOI: 10.1016/j.ijporl.2012.02.030.
6. *Bere Z, Vass G, Perenyi A, Tobias Z, Rovo L.* Surgical Solution for the Transformation of the Percutaneous Bone Anchored Hearing Aid to a Transcutaneous System in Complicated Cases. J Int Adv Otol 2020; 16(3): 477–81. DOI: 10.5152/iao.2020.6881.
7. *Hougaard DD, Boldsen SK, Jensen AM, Hansen S, Thomassen PC.* A multicenter study on objective and subjective benefits with a transcutaneous bone-anchored hearing aid device: first Nordic results. Eur Arch Otorhinolaryngol 2017; 274(8): 3011–9. DOI: 10.1007/s00405-017-4614-8.
8. *Iseri M, Orhan KS, Tuncer U, Kara A, Durgut M, Guldiken Y,* et al. Transcutaneous bone-anchored hearing aids versus percutaneous ones: multicenter comparative clinical study. Otol Neurotol 2015; 36(5): 849–53. DOI: 10.1097/MAO.0000000000000733.
9. *Carr SD, Moraleda J, Procter V, Wright K, Ray J.* Initial UK Experience with a novel magnetic transcutaneous bone conduction device. Otol Neurotol 2015; 36(8): 1399–402. DOI: 10.1097/MAO.0000000000000830.
10. *Briggs R, Van Hasselt A, Luntz M, Goycoolea M, Wigren S, Weber P,* et al. Clinical performance of a new magnetic bone conduction hearing implant system: results from a prospective, multicenter, clinical investigation. Otol Neurotol 2015; 36(5): 834–41. DOI: 10.1097/MAO.0000000000000712.
11. *Dolhen P, Lipski S, Touijar R, Van Bogaert J.* Minimal invasive pocket technique for magnet bone implant hearing aid without fixation. Eur Arch Otorhinolaryngol 2020; 277(3): 715–25. DOI: 10.1007/s00405-019-05746-5.
12. *Vaughan-Christensen L, Reed K, Smith-Olinde L.* Audiological outcomes utilizing a transcutaneous ossseointegrated implant system in pediatric patients. J Otolaryngol ENT Res 2019; 11(1): 90–5. DOI: 10.15406/joentr.2019.11.00415.
13. *Baker S, Centric A, Chennupati SK.* Innovation in abutment-free bone-anchored hearing devices in children: Updated results and experience. Int J Pediatr Otorhinolaryngol 2015; 79(10): 1667–72. DOI: 10.1016/j.ijporl.2015.07.021.
14. *Powell HR, Rolfe AM, Birman CS.* A comparative study of audiologic outcomes for Two transcutaneous bone-anchored hearing devices. Otol Neurotol 2015; 36(9): 1525–31. DOI: 10.1097/MAO.0000000000000842.
15. *Shokri T, Czarnecki B, Baker A, Isildak H.* Hearing Rehabilitation Implementing a Transcutaneous Bone Conduction Device: Single-Center Experience. Ear Nose Throat J 2021; 100(3 suppl): 199S–203S. DOI: 10.1177/0145561319870481.
16. *Clamp PJ, Briggs RJ.* The cochlear BAHA 4 attract system— design concepts, surgical technique and early clinical results. Expert Rev Med Devices 2015; 12(3): 223–30. DOI: 10.1586/17434440.2015.990375.
17. *Robinette K, Sims J, Pang B, Babu S.* Transcutaneous versus percutaneous bone-anchored hearing aids: a quality of life comparison. Am J Otolaryngol 2023; 44(2): 103758. DOI: 10.1016/j.amjoto.2022.103758.
18. *Costa JR, Costa S, Soares T, Feliciano T, Almeida E, Sousa C,* et al. Skin and soft tissue complications of bone-anchored hearing aids: Introducing a new classification system. Acta Otorrinolaringol Esp (Engl Ed) 2023; 74(2): 85–92. DOI: 10.1016/j.otoeng.2022.01.004.

Received on December 25, 2025
Revised on April 8, 2026
Accepted on April 29, 2026
Online First July 2026

CASE REPORT

UDC: 616.284-07/-08:616.28-008.14-053.6
DOI: https://doi.org/10.2298/VSP260212040C

Bilateral congenital cholesteatoma presenting as a gradual-onset hearing loss in a young adult

Obostrani kongenitalni holesteatom ispoljen kao postepeni gubitak sluha kod mlade odrasle osobe

Ljiljana Čvorović*†, Silvana Babić†, Maša Petrović‡

***University of Belgrade, Faculty of Medicine, Belgrade, Serbia; †University Clinical Center of Serbia, Clinic for Otorhinolaryngology and Maxillofacial Surgery, Belgrade, Serbia; ‡Institute for Cardiovascular Diseases "Dedinje", Belgrade, Serbia**

Abstract

Introduction. Congenital cholesteatoma (CC) is a rare pathological condition of the middle ear characterized by keratinizing squamous epithelium located behind an intact tympanic membrane. Bilateral involvement is exceptionally uncommon and may present a significant diagnostic challenge, particularly in the absence of inflammatory symptoms. **Case report.** We present the case of a 19-year-old male with a 4-year history of gradually progressive bilateral hearing loss accompanied by tinnitus and otalgia, without otorrhea or prior otologic disease. Otomicroscopy revealed a pearly white mass visible through intact tympanic membranes bilaterally. Pure-tone audiometry identified severe bilateral conductive hearing loss. Multislice computed tomography of the temporal bones revealed bilateral soft-tissue masses within the middle ear cavities, along with associated ossicular erosion. Bilateral tympanomastoidectomy with ossiculoplasty was performed, and CC occupying the mesotympanum and epitympanum was confirmed intraoperatively. Postoperative follow-up with non-echo-planar diffusion-weighted magnetic resonance imaging, performed 2 years after surgery, showed no evidence of residual or recurrent disease. **Conclusion.** This case emphasizes the importance of considering CC in adolescents and young adults presenting with bilateral severe conductive hearing loss and intact tympanic membranes. Advanced imaging is essential for accurate diagnosis and timely surgical management, even in the absence of classic inflammatory signs.

Keywords:
adult; cholesteatoma, congenital; diagnosis; hearing loss, bilateral; magnetic resonance imaging; otorhinolaryngologic surgical procedures.

Apstrakt

Uvod. Kongenitalni holesteatom (*congenital cholesteatoma* – CC) je retko patološko stanje srednjeg uva, koje odlikuje keratinizujući skvamozni epitel iza očuvane bubne opne. Obostrana zahvaćenost je izuzetno retka i može predstavljati značajan dijagnostički izazov, posebno u odsustvu simptoma zapaljenja. **Prikaz bolesnika.** Prikazujemo slučaj 19-godišnjeg muškarca sa postepenim, progresivnim, obostranim gubitkom sluha u trajanju od 4 godine, praćenog tinitusom i otalgijom, bez otoreje i prethodnih otoloških oboljenja. Otomikroskopskim pregledom viđena je biserno bela masa iza očuvane bubne opne, obostrano. Tonalnom audiometrijom utvrđen je težak obostrani konduktivni gubitak sluha. Multislajsnom kompjuterizovanom tomografijom temporalnih kostiju otkrivene su obostrane mekotkivne mase u šupljinama srednjeg uva, uz eroziju slušnih koščica. Urađena je obostrana timpanomastoidektomija sa osikuloplastikom, pri čemu je intraoperativno potvrđen CC, koji je zahvatao mezotimpanum i epitimpanum. Postoperativnim praćenjem *non-echo*-planarnom magnetnorezonantnom sekvencom difuzionog kretanja, obavljenim 2 godine posle operacije, nisu nađeni dokazi za rezidualnu ili rekurentnu bolest. **Zaključak.** Prikazom se naglašava značaj razmatranja CC kod adolescenata i mladih odraslih osoba koje imaju obostrani, težak konduktivni gubitak sluha i očuvane bubne opne. Napredno snimanje neophodno je za tačnu dijagnozu i blagovremeno hirurško lečenje, čak i u odsustvu klasičnih znakova zapaljenja.

Ključne reči:
odrasle osobe; holesteatom, urođeni; dijagnoza; sluh, gubitak, obostrani; magnetna rezonanca, snimanje; hirurgija, otorinolaringološka, procedure.

Correspondence to: Maša Petrović, Institute for Cardiovascular Diseases "Dedinje", Heroja Milana Tepića 1, 11 000 Belgrade, Serbia. E-mail: 5rovicmasa@gmail.com

Introduction

Congenital cholesteatoma (CC) is defined as a complex, multilayered, and locally invasive accumulation of stratified, keratinizing squamous epithelium located within the middle ear space behind an intact tympanic membrane. Its reported incidence is approximately 26.44 cases *per* 100,000 live births [1, 2]. Despite extensive investigation, the exact etiology of CC remains controversial, and several theories have been proposed to explain its origin [1]. The most widely accepted hypothesis suggests that CC originates from embryonic epithelial rests that fail to regress during fetal development, particularly at the time of neural groove closure. Abnormal epithelial invagination, persistence of epithelial remnants within the middle ear or antrum, or disturbances in the development of the auditory apparatus may ultimately lead to the formation of keratinized epithelial masses [1, 3].

In the majority of cases, CC is unilateral, whereas bilateral involvement is rare and has been documented only in a limited number of reports [3–7]. Clinically, CC most commonly presents in preschool-aged children with progressive conductive hearing loss (HL), often accompanied by a history of recurrent or persistent acute otitis media. A clear male predominance has been reported, with a male-to-female ratio of approximately 3 : 1. Notably, CC may also be identified in patients without a history of otorrhea, middle ear infection, tympanic membrane perforation, or prior otologic surgery, which may contribute to delayed diagnosis [8].

Otoscopic examination typically reveals a characteristic pearly white mass visible through an intact tympanic membrane, most commonly located in the anterosuperior quadrant [1, 8]. These findings distinguish CC from acquired cholesteatoma, as CC is not associated with tympanic membrane retraction and does not originate from or extend into the external auditory canal. Progressive expansion of the lesion toward the ossicular chain may occur, frequently resulting in conductive HL secondary to ossicular erosion or discontinuity [1]. High-resolution temporal bone computed tomography (CT) commonly demonstrates well-pneumatized mastoid air cells with soft-tissue opacities confined to the middle ear cavity [8].

From a diagnostic standpoint, it is essential to consider and exclude other conditions with overlapping clinical features and variable disease courses, including otitis media with effusion (OME), granulomatous middle ear disease, cholesterol granuloma, and middle ear osteoma [9–13]. Surgical excision remains the treatment of choice for CC; however, complete removal can be technically demanding, particularly in cases with ossicular involvement, where preservation of the ossicular chain poses a significant surgical challenge [8]. Owing to its rarity and often subtle clinical presentation, bilateral CC represents a distinct diagnostic entity of particular clinical interest.

Case report

A 19-year-old male was referred to our clinic with a 4-year history of gradually progressive bilateral HL. The patient additionally reported bilateral tinnitus and progressively worsening otalgia. He denied any episodes of otorrhea. His past medical history was otherwise unremarkable, and he reported no history of childhood head and neck diseases, including recurrent middle ear infections.

Otomicroscopic examination revealed a whitish, pearly appearance visible through intact tympanic membranes bilaterally (Figure 1). Pure-tone audiometry (PTA) demonstrated severe bilateral conductive HL (62.5 dB in the right ear and 55 dB in the left ear). Tympanometric assessment revealed a type B tympanogram on both sides.

Non-contrast multislice CT (MSCT) of the temporal bones demonstrated bilateral soft-tissue, mass-like densities occupying the middle ear cavities, accompanied by erosive changes of the auditory ossicles. In addition, inflammatory material was noted within the mastoid antrum and mastoid air cells, more pronounced on the left side (Figure 2).

Given the severity of HL and the risk of progressive disease-related complications, surgical intervention was indicated. Bilateral tympanomastoidectomy with ossiculoplasty was performed, revealing cholesteatoma occupying the mesotympanum and epitympanum on both sides. Intraoperatively, the tympanic membranes were found to be intact.

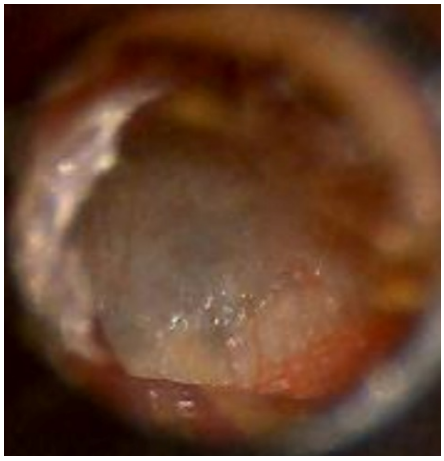

Fig. 1 – Otomicroscopy finding in the left ear pointed out a whitish glow through an intact eardrum.

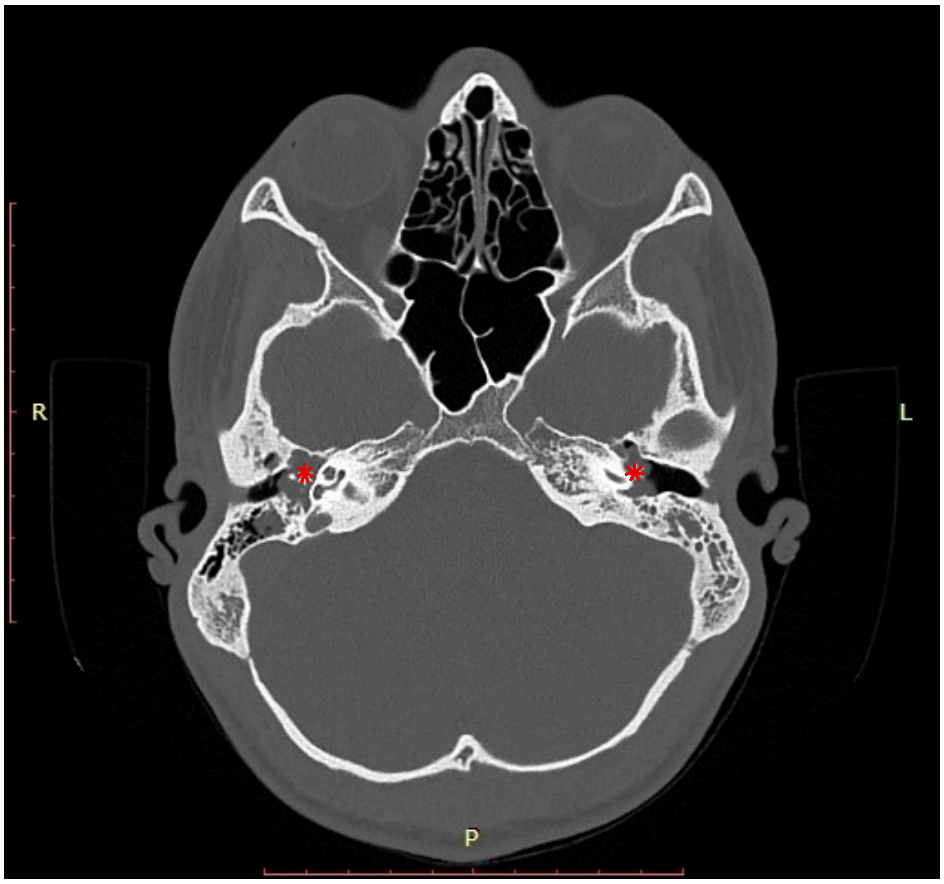

Fig. 2 – Multislice computed tomography of the temporal bones revealing a soft-tissue "mass-like" density in the middle ear cavities (red asterisk) with erosive changes on the auditory ossicles on both sides.

Due to the absence of clinical indicators of recurrence, follow-up imaging was performed 2 years postoperatively. Non-echo-planar diffusion-weighted magnetic resonance imaging (non-EPI DWI MRI) demonstrated no evidence of residual or recurrent disease.

Discussion

The present case illustrates an unusual presentation of bilateral CC in a young adult with intact tympanic membranes and no prior otologic history. The absence of recurrent otitis media, otorrhea, otologic trauma, or previous ear surgery is consistent with the diagnostic criteria for CC and likely contributed to delayed recognition of the disease. Progressive bilateral conductive HL was the dominant clinical manifestation, emphasizing the insidious nature of CC and its potential to remain undiagnosed until advanced stages.

The severity of HL observed in this patient prompted careful consideration of alternative diagnoses. While bilateral middle ear pathology with intact tympanic membranes initially suggested OME, audiometric findings demonstrated severe conductive HL, with a PTA of 62.5 dB in the right ear and 55 dB in the left ear. This is substantially greater than the degree of HL typically associated with OME. HL in OME generally ranges from 18 to 35 dB and is classified as mild conductive HL [10]. This discrepancy necessitated further diagnostic evaluation and ultimately led to exclusion of OME as a likely diagnosis.

Bilateral CC is an exceptionally rare otologic entity, with few documented cases in the literature, thereby limiting the availability of robust, high-level evidence and largely confining current understanding to case reports and small case series. Epidemiologically, bilateral CC predominantly affects the pediatric population, with a mean age at diagnosis of approximately 7.77 years and demonstrates a marked male predominance [3, 8]. Clinically, the condition most frequently presents as an incidental finding during routine otoscopic examination, although conductive HL also remains a common manifestation, underscoring the need for systematic bilateral ear assessment. In contrast to unilateral CC, bilateral cases often exhibit asymmetry, which in turn necessitates individualized and staged surgical management strategies for each ear [3]. While lesions are most commonly situated within the anterosuperior quadrant of the middle ear, more advanced presentations with mastoid extension have also been reported [14–16]. Surgical intervention remains the cornerstone of management, typically involving tympanomastoidectomy utilizing canal wall up or canal wall down techniques, with increasing integration of endoscopic approaches aimed at minimizing morbidity and improving visualization. Importantly, bilateral CC necessitates the critical importance of meticulous long-term surveillance [16]. Furthermore, the potential for sequential or delayed contrala-

teral involvement necessitates vigilant follow-up of both ears, even in cases initially presumed to be unilateral [3]. Within this context, the present case contributes to the limited existing body of evidence by further elucidating the clinical variability, diagnostic challenges, and therapeutic considerations inherent to this rare condition, reinforcing the imperative for early detection and a tailored, bilateral management approach.

Granulomatous disease of the middle ear represents another rare but important differential diagnosis in such presentations [11]. Otologic manifestations may include benign granulomatous lesions of the temporal bone, which can cause bone destruction and diagnostic uncertainty, occasionally presenting with sensorineural HL or facial nerve palsy [17, 18]. In the present case, the absence of sensorineural HL, facial nerve dysfunction, and systemic manifestations reduced the likelihood of this diagnosis, despite the presence of intact tympanic membranes, which could otherwise obscure underlying pathology.

MSCT revealed bilateral soft-tissue, mass-like opacities within the middle ear cavities, accompanied by ossicular erosion, but did not provide definitive confirmation of cholesteatoma. MSCT remains an essential imaging modality in the assessment of middle ear disease due to its excellent spatial resolution and ability to delineate bony anatomy and erosive changes. However, its capacity to characterize soft-tissue lesions is limited without contrast enhancement, often necessitating complementary imaging. In this context, magnetic resonance imaging, particularly diffusion-weighted imaging, plays a critical role in confirming the diagnosis and in postoperative surveillance [19].

Bilateral tympanomastoidectomy with ossiculoplasty was performed, revealing cholesteatoma occupying the mesotympanum and epitympanum on both sides. Intraoperatively, the tympanic membranes were found to be intact, a feature that likely masked the underlying pathology and contributed to delayed diagnosis. Postoperative follow-up is crucial in patients with cholesteatoma due to the risk of residual or recurrent disease. Current guidelines recommend diffusion-weighted MRI at intervals of 1 to 2 years, depending on clinical suspicion and patient-specific factors. In the present case, due to the absence of recurrence of clinical indicators, follow-up imaging was performed 2 years postoperatively. Non-EPI DWI MRI showed no evidence of residual or recurrent disease. This imaging modality is currently favored for postoperative surveillance owing to its high sensitivity and specificity, frequently exceeding 90% for detecting recurrent cholesteatoma [20].

Conclusion

This case highlights the need for a high index of suspicion for congenital cholesteatoma in adolescents and young adults presenting with bilateral severe conductive hearing loss, intact tympanic membranes, and absence of otorrhea. In such clinical scenarios, advanced imaging with multislice computed tomography and/or magnetic resonance imaging is essential for identifying underlying middle ear pathology and for distinguishing congenital cholesteatoma from more common conditions such as otitis media with effusion, thereby facilitating timely surgical management and preventing irreversible hearing loss and disease-related complications.

REFERENCES

1. *Pinzas L, Glaun M, Liu YC.* Congenital cholesteatoma in identical twins. Int J Pediatr Otorhinolaryngol 2022; 162: 111330. DOI: 10.1016/j.ijporl.2022.111330.
2. *Kadowaki Y, Ide S, Nakamura T, Okuda T, Shigemi H, Hirano T,* et al. Epidemiology of Congenital Cholesteatoma: Surveys of the Last 17 Years in Japan. J Clin Med 2024; 13(5): 1276. DOI: 10.3390/jcm13051276.
3. *Nourizadeh N, Afzalzadeh MR, Rashed MM.* Bilateral congenital cholesteatoma: a case report. Gaz Egypt Paediatr Assoc 2023; 71(1): 44. DOI: 10.1186/s43054-023-00195-6.
4. *Lin V, Daniel S, James A, Friedberg J.* Bilateral cholesteatomas: the hospital for sick children experience. J Otolaryngol 2004; 33(3): 145–50. DOI:10.2310/7070.2004.03023.
5. *Litman RS, Smouha E, Sher WH, Shangold LM.* Two cases of bilateral congenital cholesteatoma--usual and unusual presentations. Int J Pediatr Otorhinolaryngol 1996; 36(3): 241–52. DOI: 10.1016/0165-5876(96)01337-7.
6. *Aimi K.* Role of the tympanic ring in the pathogenesis of congenital cholesteatoma. Laryngoscope 1983; 93(9): 1140–6. DOI: 10.1288/00005537-198309000-00005.
7. *Ries M, Kostić M, Ajduk J, Trotić R, Bedeković V.* A case of bilateral congenital middle ear cholesteatoma. Braz J Otorhinolaryngol 2017; 83(6): 723–5. DOI:10.1016/j.bjorl.2015.09.003.
8. *Gilberto N, Custodio S, Colaço T, Santos R, Sousa P, Escada P.* Middle ear congenital cholesteatoma: systematic review, meta-analysis and insights on its pathogenesis. Eur Arch Otorhinolaryngol 2020; 277(4): 987–98. DOI: 10.1007/s00405-020-05792-4.
9. *Kocygit M, Ortekin SG, Cakabay T, Ozkaya G, Bezgin SU, Adali MK.* Frequency of serous otitis media in children without otolaryngological symptoms. Int Arch Otorhinolaryngol 2017; 21(2): 161–4. DOI: 10.1055/s-0036-1584362.
10. *Cai T, McPherson B.* Hearing loss in children with otitis media with effusion: a systematic review. Int J Audiol 2016; 56(2): 65–76. DOI:10.1080/14992027.2016.1250960.
11. *Rossini AA, Bogaz A, Yonamine K, Testa RG, Penido Nde O.* Refractory otitis media as the first manifestation of Wegener's granulomatosis. Braz J Otorhinolaryngol 2010; 76(4): 541. DOI: 10.1590/S1808-86942010000400024.
12. *Ahmetgjekaj I, Harizi E, Rahman A, Hyseni F, Nasir F, Decka A,* et al. Giant cholesterol granuloma of petrous apex. Radiol Case Reports 2022; 17(4): 1220–4. DOI: 10.1016/j.radcr.2021.12.046.
13. *Marţu C, Cozma S, Cobzeanu B, Vesa D, Butnaru C, Bularda D,* et al. Serous otitis media: Clinical and therapeutic considerations, including dexamethasone (C22H29FO5) intratympanic injection. Exp Ther Med 2022; 23(2): 125. DOI: 10.3892/etm.2021.11048.
14. *Cho HS, Kim HG, Jung DJ, Jang JH, Lee SH, Lee KY.* Clinical Aspects and Surgical Outcomes of Congenital Cholesteatoma in 93 Children: Increasing Trends of Congenital Cholesteatoma from 1997 through 2012. J Audiol Otol 2016; 20(3): 168–73. DOI: 10.7874/jao.2016.20.3.168.
15. *Klopper GJ, Favara C.* Synchronous bilateral idiopathic external auditory canal cholesteatoma: a case report and review of the literature. Egypt J Otolaryngol 2023; 39(1): 98. DOI: 10.1186/s43163-023-00459-3.

16. *El-Bitar MA, Choi SS*. Bilateral Occurrence of Congenital Middle Ear Cholesteatoma. Otolaryngol Head Neck Surg 2002; 127(5): 480–2. DOI: 10.1067/mhn.2002.128898.
17. *Rezende EB, Rodrigues EC, Yoshimura R, Uvo P, Rapoport B*. Wegener's granulomatosis: a case report. Rev Bras Otorrinolaringol 2003; 69(2): 261–5. DOI:10.1590/S0034-72992003000200018.
18. *Iannella G, Stasolla A, Pasquariello B, Re M, Magliulo G*. Tympanomastoid cholesterol granuloma: radiological and intraoperative findings of blood source connection. Eur Arch Otorhinolaryngol 2016; 273(9): 2395–401. DOI: 10.1007/s00405-015-3820-5.
19. *Swartz JD, Harnsberger HR*. Imaging of the Temporal Bone. 3rd ed. Thieme-Stratton Corp; 2003. 509 p.
20. *Zaman SU, Rangankar VP, Krishnarjun M, Kalekar TM, Shah VP, Pawar R,* et al. Readout-Segmented Echoplanar (RESOLVE) Diffusion-Weighted Imaging on 3T MRI in Detection of Cholesteatoma—Our Experience. Indian J Radiol Imaging 2023; 34(1): 16–24. DOI: 10.1055/s-0043-1776054.

Received on February 12, 2026
Revised on March 1, 2026
Revised on May 1, 2026
Accepted May 13, 2026
Online First July, 2026

INSTRUCTIONS FOR AUTHORS

Before submitting a manuscript for consideration for publication in the journal *Vojnosanitetski pregled* (VSP), authors are required to carefully read the Instructions for Authors in order to prepare the manuscript in accordance with the journal's guidelines.

A manuscript that does not meet the requirements of these instructions cannot be considered and will be returned to the authors for completion and correction.

Upon acceptance of the manuscript for publication in VSP, the authors transfer their copyright to the journal's publisher, the Ministry of Defense of the Republic of Serbia, University of Defense.

VSP adheres to the recommendations of the International Committee of Medical Journal Editors (ICMJE), *Recommendations for the Conduct, Reporting, Editing, and Publication of Scholarly Work in Medical Journals* (available at https://www.icmje.org/recommendations/).

VSP is available in open access mode. All articles may be downloaded free of charge from the journal's website and used in accordance with the Creative Commons Attribution–ShareAlike (CC BY-SA) license (https://creativecommons.org/licenses/by-sa/4.0/deed.en).

SENDING MANUSCRIPTS

The manuscript and all accompanying materials should be submitted **as a single document** (with all appendices incorporated into the text and placed at the end of the manuscript after the References section), exclusively in electronic form *via* the Aseestant submission system. In order to preserve quality, submitting the images as separate files is also recommended to avoid image compression and potential loss of quality, since Word may reduce their resolution. All authors and reviewers must be registered system users with a unique email address. Registration is possible at: http://aseestant.ceon.rs/index.php/vsp/user. The technical guide for using the electronic submission system is available at: https://aseestant.ceon.rs/index.php/vsp/about/submissions.

If you encounter any problems submitting your manuscript *via* the Aseestant platform, you can contact the journal's Editorial Office for assistance by sending an email to: vsp@vma.mod.gov.rs.

GENERAL INSTRUCTIONS

VSP publishes manuscripts that have not been previously published, in whole or in part, and that are not under consideration for publication or have not been accepted for publication in any other journal.

VSP does not consider manuscripts that have been previously published as preprints.

The journal accepts manuscripts whose results have been previously presented at scientific or professional meetings and published as abstracts, provided that these results have not been published with a DOI (e.g., an extended abstract in a journal supplement).

If part of the results presented in the submitted manuscript has previously been reported at a scientific/professional meeting or is part of a doctoral dissertation, the Submission Letter to the Editorial Board must specify the official name of the meeting, its location and date, and indicate whether the presented results have been published and in what form (e.g., same or different title or abstract). This information should also be clearly stated in a Note at the end of the manuscript.

Manuscripts are published in English. Certain categories of articles (e.g., history of medicine/dentistry/pharmacy) may, at the discretion of the VSP Editorial Board, be published in Serbian. All manuscript categories, except for Editorials, Letters to the Editor, Research Letters, Book Reviews, and Reports from Scientific or Professional Meetings, must include abstracts in both Serbian and English (as part of the manuscript). For details on the structure and length of the abstract, see the Abstract section of these Instructions.

Manuscripts should be prepared using Microsoft Word, with Times New Roman font, size 12 pt, and line spacing 1.5. Set the page size to A4, with a left margin of 4 cm and the remaining three margins 2 cm each. Text should be typed without hyphenation, and only one space should follow each punctuation mark. If special characters (symbols) are used in the text, the Symbol font should be applied.

References cited in the text should be indicated with Arabic numerals in superscript, in the order in which they appear.

Pages should be numbered consecutively in the bottom right corner, starting from the first page (excluding the title page).

When writing in English, follow the American English language standard. The International System of Units (SI) must be used, except for blood pressure (mm Hg) and temperature (°C).

Use standard abbreviations throughout the text. Avoid abbreviations in the title and abstract unless necessary. At their first mention, provide the full term followed by the abbreviation in parentheses; thereafter, use only the abbreviation in both the abstract and the main text. Do not use abbreviations in the Conclusion section (excluding the abstract).

Do not use commercial names of drugs or other products; if necessary, the generic name must also be provided. Devices and equipment should be referred to by their trade names, with manufacturer details (name and location) provided in round brackets. If labels combining letters and numbers are used in the text, ensure that superscript or subscript numbers are written precisely.

Avoid using bold or italic fonts, as they are reserved for subheadings. Exceptions include terms that must be italicized, such as gene names or foreign words in Latin.

Study groups must be clearly defined and consistently named throughout the manuscript. Use a single, consistent term for the same concept. In the Results section, avoid sentences beginning with phrases like "Table X shows" or "Figure X illustrates". Instead, the sentence should describe the result, with the table or figure reference placed in parentheses at the end of the sentence. Sentences should not begin with abbreviations, numbers, or dates. Avoid overly long sentences that reduce clarity; favor short, clear sentences. The Conclusion should be written with new sentences, without repeating previously stated ones. Translation of manuscripts into English using Google Translate may lead to misunderstandings and is therefore not recommended.

When selecting keywords, use Medical Subject Headings – MeSH (https://www.nlm.nih.gov/mesh/meshhome.html). Keywords in the accepted manuscript cannot be altered by the authors, as they are descriptors from the Thesaurus used by professional indexers.

REQUIRED ACCOMPANYING DOCUMENTS

AUTHOR STATEMENT AND AUTHORSHIP

For every manuscript submitted for consideration for publication in VSP, the author(s) must provide an Authorship Statement Form (ASF) confirming that the work has not been previously published and is not simultaneously under consideration for publication in another journal. The ASF must also confirm that all authors meeting the authorship criteria have read and approved the manuscript, and provide contact information for all authors (email address and mobile phone number). In this form, authors must declare any potential conflicts of interest or the absence thereof. All authors must sign the ASF by hand.

For additional information on different types of conflicts of interest, see the recommendations of the World Association of Medical Editors – WAME: http://www.wame.org.

VSP follows the authorship criteria recommended by ICMJE (https://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html). Authorship is based on fulfilling all four given criteria: substantial contributions to the conception of the work, acquisition of data or analysis/interpretation of data; critical revision of the manuscript for important intellectual content; approval of the final version of the manuscript to be published; accountability for all aspects of the published work. All other contributors who participated in the work but do not meet the authorship criteria should be listed in the Acknowledgements, specifying their contribution. Individuals mentioned in the Acknowledgements must provide written consent.

ETHICAL APPROVAL

All research involving humans and/or human material must be conducted in accordance with the ICMJE recommendations (https://www.icmje.org/recommendations/browse/roles-and-responsibilities/protection-of-research-participants.html) and the Declaration of Helsinki, 2024 revision (https://www.wma.net/policies-post/wma-declaration-of-helsinki/). Authors are required to submit a scanned copy of the Ethics Committee (EC) approval from the competent institution that approved the study, showing the date of issuance and the subject of the research, along with the manuscript. The EC approval should be submitted in the original language and in English (a certified copy is acceptable).

The Methods section must indicate that the study was approved by the relevant EC, including the name of the institution and the approval number, and that it was conducted in accordance with ethical principles for research involving humans and/or human material.

Patient anonymity must be protected following ICMJE recommendations. For research involving patient data that could allow direct or indirect identification, authors must obtain written informed consent from the patient, state in the manuscript that consent was obtained, and provide it to the Editorial Board if requested.

For research involving animals, authors must provide approval from the relevant EC ensuring compliance with international standards for the use of laboratory animals in research.

The Editorial Board reserves the right to reject manuscripts deemed not conducted in accordance with international ethical standards.

REPRODUCTION OF PREVIOUSLY PUBLISHED OR UNPUBLISHED THIRD-PARTY COPYRIGHTED MATERIAL

If previously published illustrations (photographs, diagrams, etc.) are used, the source must be cited, and permission must be obtained from the journal in which they were originally published, granting approval for their use in VSP. If unpublished third-party illustrations (photographs, diagrams, etc.) are used, permission must be obtained from the original author(s) for publication in VSP.

PLAGIARISM

Since 2012, all manuscripts submitted to VSP are checked for potential (self-)plagiarism using SCIndeks Assistant – Cross Check (iThenticate). Manuscripts found to contain (self-)plagiarism will be rejected. Depending on the extent and type of detected (self-)plagiarism, authors may be banned from publishing in VSP for varying durations. Relevant authorities at the authors' institutions and appropriate professional associations will also be notified.

USE OF AI

Generative artificial intelligence (AI) or AI-assisted technologies may be used only in compliance with the principles of transparency (the use of AI must be clearly stated in the manuscript), accountability (authors remain fully responsible for the accuracy and originality of the content), verifiability (all participants in the publishing process must verify that AI has not introduced fabricated data, citations, or claims), and confidentiality (authors and reviewers are prohibited from uploading manuscripts submitted to VSP to public AI services).

The use of AI tools is permitted only for limited linguistic and technical interventions in the manuscript text: grammar and spelling correction, stylistic refinement of the authors' text, assistance with formatting, and technical assistance (such as code correction). Authors may use AI tools exclusively to create AI-assisted, but not AI-generated content.

Authors who have used AI-assisted content are required to fully and accurately disclose the use of AI tools (the exact name of the AI tool, date of access, prompts used, and purpose of use), guarantee the originality of the

scientific contribution, avoid any fabrication or manipulation, and comply with the rules of scientific ethics. Information on AI use should be stated in the Methods or Acknowledgements section.

Using AI tools is prohibited for the following: generating substantial portions of the manuscript content; creating scientific ideas, data, or results; analyzing or interpreting results; formulating conclusions; modifying images, tables, or graphs (including graphical abstracts); altering data or references.

Any unequivocally established improper use of AI will result in rejection of the manuscript.

AI may not under any circumstances be an author or co-author, nor may it be cited as an author in the References section.

To protect confidentiality, no part of unpublished research submitted to VSP may be entered into a large language model by authors or reviewers.

Authors who have used any AI tools are required to submit an AI Use Statement when submitting the manuscript.

TYPES OF MANUSCRIPTS

VSP publishes the following categories and types of manuscripts and communications: Editorial, Original Article, Preliminary Report, Short Report, Case Report and Case Series, General (Narrative) Literature Review, Mini-Review, Systematic Literature Review, Meta-Analysis, Systematic Literature Review with Meta-Analysis, Current Topic, In Focus, Article on the History of Medicine/Dentistry/Pharmacy, Letter to the Editor, Research Letter, Clinical Research, Congress and Scientific Meeting Report, Book Review, In Memoriam, and other contributions.

ORIGINAL ARTICLE

An Original Article presents new and significant findings in a specific field, with a detailed description of the research methods used, the results obtained, and the conclusions drawn. The reference list should include the most recent and most relevant references in the field.

PRELIMINARY REPORT

A Preliminary Report presents research that has not yet been completed, with findings that require further investigation and validation before final conclusions can be drawn, but where the obtained information is of interest to the scientific and professional community. It contains all sections of an Original Article, but in a substantially abbreviated form. Authors are encouraged to subsequently publish a full Original Article with complete, validated data and a comprehensive analysis.

SHORT REPORT

A Short Report presents a completed research study that is small in scope, narrowly focused, and has clear conclusions based on the presented results. It includes all sections of an Original Article, but in a substantially abbreviated form. It is considered the final publication of that specific, limited study and cannot be republished as a full-length article (although follow-up research building on it is encouraged).

REVIEW ARTICLES

GENERAL (NARRATIVE) LITERATURE REVIEW

A General (Narrative) Literature Review provides a review, critical analysis, and synthesis of existing scientific knowledge on a selected topic. Authors cover all available relevant literature over a defined time period, present the results of relevant studies, identify gaps, limitations, or controversies, and indicate directions for future research, offering their own perspective on the issue in the form of concluding remarks.

Authors of this category of article should have published at least five papers in peer-reviewed journals (M20) in the field of the review topic.

MINI-REVIEW ARTICLE

A Mini-Review provides a concise overview of the existing literature and the most recent advances within defined aspects of a particular research field, as well as its new and/or current directions of development.

SYSTEMATIC LITERATURE REVIEW

A Systematic Literature Review synthesizes previously published studies on a specific topic using clearly defined and pre-established methodological procedures for study selection and evaluation. The author must use relevant databases, define inclusion and exclusion criteria, and apply a transparent methodology.

META-ANALYSIS

A Meta-Analysis uses statistical methods to combine quantitative data from multiple primary studies in order to identify overall trends and assess the strength of evidence on a specific topic. Authors must use relevant databases, define inclusion and exclusion criteria, and apply a transparent and reproducible methodology. The research question must be clearly defined using the PICOS framework, and selection guidelines and a study flow diagram (PRISMA) must be provided.

SYSTEMATIC LITERATURE REVIEW WITH META-ANALYSIS

A Systematic Literature Review with Meta-Analysis combines qualitative and quantitative synthesis, using statistical techniques to summarize quantitative results and qualitative synthesis for descriptive/narrative findings. Authors must use relevant databases, clearly define inclusion and exclusion criteria, and apply a transparent and reproducible methodology. The research question must be clearly defined according to the PICOS framework, with specification of the reporting guidelines used (e.g., PRISMA) and inclusion of a PRISMA flow diagram showing study selection.

CURRENT TOPIC

A Current Topic addresses a contemporary, unresolved, or controversial issue of theoretical and practical importance, presenting the authors' own research results or the most recent important data from the literature. The structure of the article is flexible, and brief concluding remarks with a clear message are encouraged.

IN FOCUS

An In Focus article provides a thematic, focused analysis or a brief overview of a scientific issue within the journal's scope, addressing a topic of significance for the scientific community and broader professional audience.

CASE REPORTS

CASE REPORT and CASE SERIES (≥4, ≤9)

Case reports or case series present cases with rare or unusual diagnoses, diagnostic processes, treatment strategies, clinical courses, or treatment outcomes that may be useful for clinical practice and medical education. The CARE guidelines should be followed when preparing the manuscript (https://www.care-statement.org/writing-a-case-report). Written informed consent from the patient is mandatory.

EDITORIAL

Editorials are non–peer-reviewed texts written by the Editor-in-Chief and/or members of the Editorial Board, intended to announce a new volume, special issues, or content of significance for the profession and/or institutions served by the journal, as well as invited editorial texts. Editorials should not contain unpublished or original data, and must include a statement of conflict of interest.

LETTER TO THE EDITOR

A non–peer-reviewed comment or critique of a paper published in VSP. It is written in a free format, with optional citation of relevant literature, and must not contain unpublished results. It is published at the discretion of the Editor-in-Chief.

RESEARCH LETTER

A Research Letter is a short report of original research, containing Introduction, Methods, Results, and Discussion in a condensed form (without separate sections or subheadings) and up to 2 supplementary items (tables/figures). It does not include an abstract or keywords, but must meet all general manuscript requirements for consideration, including the peer-review process.

HISTORY OF MEDICINE/STOMATOLOGY/PHARMACY

Manuscripts presenting material relevant to elucidating specific events and/or portraying notable figures in the history of medicine/stomatology/pharmacy, with particular emphasis on military medicine/stomatology/pharmacy.

CLINICAL RESEARCH

Clinical Research includes original randomized controlled trials and observational studies assessing the impact of one or more interventions or measures on human health outcomes, clinical practice, or health policy.

Manuscripts must be prepared in accordance with international guidelines (e.g., CONSORT – https://www.consort-statement.org/ or STROBE – https://www.strobe-statement.org/) and be registered in a recognized public registry (e.g., ClinicalTrials.gov).

BOOK REVIEW

A Book Review includes bibliographic details of the publication (authors, original title, publisher, place, and year of publication), a brief summary, and critical comments on the content, style, and significance of the book in the relevant field. The manuscript must not exceed 2 pages.

SCIENTIFIC MEETING REPORT

A Scientific Meeting Report presents the activities of a scientific or professional meeting, highlighting the most important presentations, conclusions, or recommendations relevant to the wider readership of VSP.

MANUSCRIPT LENGTH

A complete manuscript consists of: title page, abstracts in Serbian and English with keywords, main text, acknowledgements (if applicable), reference list, and supplementary material (tables, figures, charts, diagrams, drawings).

For Original Article, General (Narrative) Literature Review, Systematic Literature Review, Meta-Analysis, and Systematic Literature Review with Meta-Analysis, the manuscript length may not exceed 5,000 words.

For Mini-Review, Preliminary Report, Short Report, Case Report, Case Series, Current Topic, Clinical Research, and History of Medicine/Stomatology/Pharmacy, the manuscript length may not exceed 3,000 words.

Manuscripts in other categories/sections may have a maximum of 1,500 words.

MANUSCRIPT PREPARATION

TITLE PAGE

The first page of the manuscript should include the following:

1. Title of the manuscript without abbreviations;
2. Full names of all authors (without academic titles, but with ORCID numbers included for those who have them) with symbols assigned in the following order: ***, †, ‡, §, ‖, ¶, **, ††**… etc.;
3. Full official names of the institutions where the authors work, including city and country of the institution (the symbols *, †, ‡, §, ‖, ¶, **, †† … etc. correspond to the institutions of each author);
4. At the bottom of the page, provide the name and surname, postal address, email address, and phone number (mobile/Viber or WhatsApp) of the author responsible for correspondence.

ABSTRACT

The abstract and keywords should be provided on the second page of the manuscript. The abstract should be written in short and clear sentences. For the

categories Original Article, Preliminary Report, Short Report, Systematic Literature Review with Meta-Analysis, Meta-Analysis, and Clinical Research, the abstract must be structured and include the following sections: Introduction/Aim, Methods, Results, Conclusion. Each section should be written as a separate paragraph beginning with a bolded heading. The most important results should be presented, including numerical values and the level of statistical significance. The conclusion must be directly related to the study results. The abstract must not exceed 300 words.

For the categories Case Report and Case Series, the abstract should have the following structure: Introduction (with the aim stated in the last sentence), Case Report, Conclusion. Each section should be written as a separate paragraph beginning with a bolded heading. The abstract must not exceed 250 words.

For all other manuscript categories: General (Narrative) Literature Review, Mini Review, Systematic Literature Review, Current Topic, In Focus, and History of Medicine/Stomatology/Pharmacy, the abstract is unstructured and must not exceed 200 words.

Care should be taken in ensuring that the Serbian and English versions of the abstract are accurate and precise translations of each other. No sentence may appear in one version without being translated into the other.

KEYWORDS

Below the abstract, list five to seven relevant keywords or phrases that indicate the content of the manuscript. It is recommended to avoid repeating words from the title of the paper. When selecting keywords, use Medical Subject Headings (MeSH) (https://www.nlm.nih.gov/mesh/meshhome.html).

STRUCTURE OF THE MAIN TEXT

Original Articles, Preliminary Reports, Short Reports, Meta-Analyses, Systematic Literature Reviews with Meta-Analysis, and Clinical Research papers must include the following sections: Introduction (a brief overview of the research topic, with the study aim stated in the final paragraph); Methods (a precise description of participant selection and applied methods, including statistical methods, and the approval number of the competent Ethics Committee); Results (presented in a logical order, without duplicating the same results in multiple forms); Discussion (without repeating data already presented in the Results section; only the obtained findings should be discussed, placing them in the context of other relevant studies; the discussion and conclusions should be linked to the study aims, and study limitations should be highlighted if necessary); Conclusion (derived directly from the study results); Acknowledgements (if applicable); References.

Manuscripts in the categories General (Narrative) Literature Review, Mini-Review, Systematic Literature Review, Current Topic, and In Focus should contain the following sections: Introduction (with appropriate subheadings), Conclusion, and References.

Manuscripts in the categories Case Report and Case Series should include the following sections: Introduction (the aim of the paper should be stated in the final paragraph of the Introduction), Case Report (the patient's identity must remain anonymous), Discussion, and References.

A Case Report must not have more than five authors.

QUESTIONNAIRES

All questionnaires used as measurement instruments for any of the investigated parameters must be translated into the language spoken by the study participants, with evidence provided of their validation and cultural adaptation to the participants' setting.

TABLES AND FIGURES

Tables and figures, the number of which should be appropriate to the length of the text, should be placed at the end of the main manuscript text, after the References. The exact position of each item should be clearly indicated in the text. The final placement of tables and figures will be determined during manuscript preparation for publication.

Tables

The title should be placed above the table, and explanations (the legend) below it. Tables should be numbered with Arabic numerals in the order in which they appear in the text. Tables must be created exclusively in the Microsoft Word program using the menu Table–Insert–Table, with the exact number of rows and columns defined. Use Times New Roman font, 12-point size, single spacing. Tables must be clear and include all elements necessary for the proper interpretation of the data presented. If the displayed values have ranges or reference values, these must be specified.

In the legend below the table, all abbreviations used in the table and all symbols (e.g., superscript letters or bolded values) must be explained. In addition, the applied statistical methods must be clearly specified.

Figures (Illustrations)

Figures include all forms of graphical material (photographs, drawings, diagrams, and graphs). Figures should be embedded in the manuscript at the end of the text, after the References and after the Tables (if any). Figures should be numbered with Arabic numerals in the order in which they appear in the text. Capital letters A, B, C, etc., should be used to designate parts of multipart figures. Letters, numbers, and symbols must be clear, consistent, and of sufficient size to remain legible after reduction. All elements shown in figures must be saved as images (not as editable graphic objects) so that their position cannot be altered, ensuring the accuracy of the data presented. Only digital images with a minimum resolution of 300 dpi and in JPEG, PNG, or PDF format are accepted. Figures that do not meet these requirements will not be accepted for publication. The dimensions of submitted figures should be approximately the same as the dimensions at which they will be published. If authors are unable to provide digital photographs, original images should be scanned at a resolution of 300 dpi and at their original size and submitted in that form. All text in diagrams and graphs should be written in a sans-serif font for better readability (e.g., Arial, Helvetica), with a font size of no less than 10 pt. Measurement units and scales must be clearly indicated. Decimal numbers in graphs must be presented with a decimal point, and thousands should be separated by a comma (e.g., 1,234.56).

Video supplements (illustrations of the manuscript) may last 1–3 minutes and should be submitted in AVI or MP4 (FLV) format. A separate still image representing the video (video thumbnail) must also be provided for use in the electronic edition and publication in the printed edition, along with a link to the platform where the video is already hosted.

In the legend below each illustration, all abbreviations, symbols, numbers, or letters used to explain individual parts of the figure must be defined. For graphs, the applied statistical methods should be specified where appropriate; for photomicrographs, details of the staining method and magnification must be provided.

If photographs of persons (patients) are presented, the face must be blurred or written consent must be obtained from the person depicted. In imaging materials (X-rays, CT scans, ultrasound images, etc.), all information that could identify the patient must be removed. If a figure has been previously published, the source must be cited, and written permission must be obtained if the material is protected by copyright.

ABBREVIATIONS

Abbreviations should be used only when necessary, primarily for very long names of chemical compounds or for terms that are already widely recognized in abbreviated form (e.g., DNA). For each abbreviation—except standard units of measurement—the full term must be given at its first occurrence in the text (including the abstract). The use of abbreviations should be avoided in the title and abstract; in the title, abbreviations should be used only if absolutely necessary. For terms mentioned more than 3 times in the text, introducing appropriate abbreviations is recommended.

DECIMAL NUMBERS

In manuscripts written in English, decimal numbers should be written with a decimal point (e.g., 22.7), whereas in manuscripts written in Serbian, a comma should be used (e.g., 22,7). Whenever possible, numbers should be rounded to one decimal place and reported consistently throughout the manuscript (e.g., if one value is 32.2, all others should also be rounded to one decimal place, e.g., 32.0).

UNITS OF MEASUREMENT

Length, height, weight, and volume should be expressed in metric units (meter – m, kilogram (gram) – kg (g), liter – L) or their subunits. Temperature should be expressed in degrees Celsius (°C), and blood pressure in millimeters of mercury (mm Hg). Results of clinical and biochemical measurements should be reported in metric units according to the International System of Units (SI).

ACKNOWLEDGEMENTS

The contributions of individuals who should be acknowledged but do not meet the criteria for authorship should be stated. Financial support (sponsorships, grants, equipment, etc.) should be disclosed, as well as the name of the project within which the research was conducted.

STATISTICAL ANALYSIS

In the Methods section, the applied statistical methods should be described in sufficient detail to allow verification of their correct use and reproduction of the analysis. Results must be presented numerically and clearly, with appropriate measures of variability and reliability (e.g., standard deviation, standard error, confidence interval). The type of study should be specified, and the manner in which it was conducted should be described. Inclusion and exclusion criteria must be stated. The software and the version of the computer program used for statistical data analysis should be reported. In the Results section, as well as in the legends of tables and/or figures, the statistical method used to analyze the presented results must be indicated. The p values should always be written with a leading zero (e.g., $p > 0.05$, not $p > .05$).

REFERENCES

References should be numbered with Arabic numerals according to the order of their first appearance in the text (including tables and figure legends). It is recommended that the majority of cited references be published within the last ten years. At least 80% of the cited references should be original research articles, while books, book chapters, and review articles should account for no more than 20% of the total number of references. All references, regardless of the language of the original source, must be cited in English, with the original language indicated in parentheses after the reference.

All data on the references must be accurate, and the cited works should be easily accessible to readers. A DOI number must be provided for each reference. Citation of articles published in journals indexed in Current Contents, Index Medicus (MEDLINE), Excerpta Medica, Scopus, and Web of Science is recommended.

Citation of abstracts, secondary publications, oral communications, unpublished works, official or confidential documents, Wikipedia, preprints and in press articles, retracted articles, and articles published in predatory journals is not permitted.

When citing websites, the homepage must not be cited; instead, the specific webpage from which the information was obtained must be referenced. Each cited reference must be available for online verification. If a reference is not available online (e.g., archival material), the author must provide the source from which the cited material was obtained, or submit a photographed or scanned copy of the document by emailing it to: strliteratura@gmail.com.

References should be formatted according to the Vancouver style established by the ICMJE (https://connect.ebsco.com/s/article/Citing-Articles-in-Vancouver-ICMJE-Style?language=en_US).

Citation examples:

Article with 1 to 6 authors

*Nikolić A, Bioč*anin *V, Rančić N, Dušpara M, Đurić D.* Serbian translation and validation of the SF-36 for the assessment of quality of life in patients with diagnosed arterial hypertension. EABR Exp Appl Biomed Res 2023; 24(3): 227–34. DOI: 10.2478/sjecr-2020-0073

Article with more than 6 authors

Kapur VK, Auckley DH, Chowdhuri S, Kuhlmann DC, Mehra R, Ramar K, et al. Clinical Practice Guideline for Diagnostic Testing for Adult Obstructive Sleep Apnea: An American Academy of Sleep Medicine Clinical Practice Guideline. J Clin Sleep Med 2017; 13(3): 479–504. DOI: 10.5664/jcsm.6506

Volume with a Supplement

Smith JA, Brown LM. Effects of vitamin D on immune response. J Nutr Sci 2024; 15(Suppl 2): S45–53.

Issue with a Supplement

Zhou Q, Shi R, Kopjar B, Wang H, Chen D, Li H, et al. Adjacent Intervertebral Disc Changes in Patients with Isobar Semirigid Dynamic Stabilization System. Global Spine J 2017; 4(1 Suppl): s-0034-1376699.

Volume with Part (Pt)

Ozben T, Nacitarhan S, Tuncer N. Plasma and urine sialic acid in non-insulin dependent diabetes mellitus. Ann Clin Biochem 1995; 32(Pt 3): 303–6.

Issue with Part (Pt)

Poole GH, Mills SM. One hundred consecutive cases of flap lacerations of the leg in ageing patients. N Z Med J 1994; 107(986 Pt 1): 377–8.

Issue with no Volume

Turan I, Wredmark T, Fellander-Tsai L. Arthroscopic ankle arthrodesis in rheumatoid arthritis. Clin Orthop 1995; (320): 110–4.

No Volume or Issue

Browell DA, Lennard TW. Immunologic status of the cancer patient and the effects of blood transfusion on antitumor responses. Curr Opin Gen Surg 1993: 325–33.

Pagination with Roman numerals

Fisher GA, Sikic BI. Drug resistance in clinical oncology and hematology. Introduction. Hematol Oncol Clin North Am 1995; 9(2): xi–xii.

Book

Printed Book

Ritter JM, Flower RJ, Henderson G, Loke YK, MacEwan D, Robinson E, et al. Rang & Dale's Pharmacology. 10th ed. London: Elsevier; 2023. p. 3630.

Book in electronic format

Shreeve DF. Reactive attachment disorder: a case-based approach [Internet]. New York: Springer; 2012 [cited 2012 Nov 2]. 85 p. Available from: http://dx.doi.org/10.1007/978-1-4614-1647-0

Chapter

In an edited book

Metcalf CS, Smith MD, Wilcox KS. Pharmacotherapy of the Epilepsies. In*: Brunton LL, Knollmann BC,* editors. Goodman & Gilman's The pharmacological basis of therapeutics. 14th ed. NY: McGrawHill; 2023. p. 385–411.

In an edited electronic (online) book

Halpen-Felsher BL, Morrell HE. Preventing and reducing tobacco use. In: *Berlan ED, Bravender T,* editors. Adolescent medicine today: a guide to caring for the adolescent patient [Internet]. Singapore: World Scientific Publishing Co.; 2012 [cited 2012 Nov 3]. Chapter 18. Available from: http://www.worldscientific.com/doi/pdf/10.1142/9789814324496_0018

Website

Homepage

Diabetes Australia. Diabetes globally [Internet]. Canberra ACT: Diabetes Australia; 2012 [updated 2012 June 15; cited 2012 Nov 2]. 85 p. Available from: http://www.diabetesaustralia.com.au/en/Understanding-Diabetes/Diabetes-Globally/

Part of a website

Australian Medical Association [Internet]. Barton ACT: AMA; c1995-2012. Junior doctors and medical students call for urgent solution to medical training crisis; 2012 Oct 22 [cited 2012 Nov 2]; [about 3 screens]. Available from: https://ama.com.au/media/junior-doctors-and-medical-students-call-urgent-solution-medical-training-crisis

Conference Proceedings

Kimura J, Shibasaki H, editors. Recent advances in clinical neurophysiology. Proceedings of the 10th International Congress of EMG and Clinical Neurophysiology; 1995 Oct 15–19; Kyoto, Japan. Amsterdam: Elsevier; 1996.

Article from Conference Proceedings

Bengtsson S, Solheim BG. Enforcement of data protection, privacy and security in medical informatics. In: *Lun KC, Degoulet P, Piemme TE, Rienhoff O,* editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sep 6–10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. p. 1561–5.

Dissertation

Knežević D. The importance of decontamination as an element of complex therapy of poisoning with organophosphorous compounds [Ph.D. Thesis]. Belgrade: School of Veterinary Medicine; 1988. (Serbian)

Other published articles

News article

Vujadinović J. The inconsistency between federal and republican regulation about pharmacies. In between double standards. Borba 2002 February 28; p. 5. (Serbian)

Holy Bible

Serbian Bible. Belgrade: British and Foreign Biblical Society; 1981. Book of Isaiah 2: 19–22. (Serbian)

Dictionaries and similar references

Kostić AĐ. Multilingual Medical Dictionary. 4th Ed. Belgrade: Nolit; 1976. Erythrophobia; p. 173–4.

Other examples of citing publications can be seen at https://www.nlm.nih.gov/bsd/uniform_requirements.html

ARTICLE PUBLICATION FEES

The publication fee is to be paid after the manuscript has been accepted for publication. Decision on accepting the manuscript must be confirmed at a VSP Editorial Board meeting. Manuscripts for which the fee has not been paid will not be published.

Payment of the fee does not guarantee that the manuscript will be published in VSP.

All authors are required to pay the Article Processing Charge to cover the costs of language editing, as well as professional and technical processing of the manuscript, and its publication. Domestic authors pay 5,000 RSD, and international authors pay 150 EUR.

Institutional subscription (10,000 RSD) applies only to institutions that wish to receive physical copies of the journal's monthly issues. In the year the subscription is paid, authors can submit an unlimited number of manuscripts to VSP.

Copies of the payment receipt must be sent to strliteratura@gmail.com for record-keeping.

The following are exempt from the subscription fee: VSP reviewers (a manuscript ID or title of the reviewed paper must be provided), doctoral students (with proof of enrollment in the current academic year), and editors. The Editorial Board reserves the right to reduce or waive the subscription fee upon a justified request from the author.

For any additional information, you can contact the addresses and phone numbers listed below.

***Vojnosanitetski pregled* Editorial Office**
17 Crnotravska St
11 000 Belgrade
Serbia
Phone number: (+381 11) 3608-997
E-mail: vsp@vma.mod.gov.rs
E-mail for subscription information: strliteratura@gmail.com
Website: www.vsp.mod.gov.rs
ISSN 0042-8450
ISSN Online 2406-0720 OPEN ACCESS

УПУТСТВО ЗА АУТОРЕ

Пре подношења рукописа за разматрање за објављивање у часопису „Војносанитетски преглед“ (ВСП) неопходно је да аутори пажљиво прочитају Упутство за ауторе, како би рукопис припремили у складу са пропозицијама часописа.

Рад који не испуњава услове овог упутства не може бити разматран и биће враћен ауторима да га допуне и исправе**.**

Аутори рада преносе своја ауторска права на издавача часописа Министарство одбране Републике Србије, Универзитет одбране **након прихватања рада за објављивање у ВСП**.

ВСП се придржава препорука Међународног комитета уредника медицинских часописа (*International Committee of Medical Journal Editors* – ICMJE), *Препоруке за спровођење, извештавање, уређивање и публиковање научних радова у медицинским часописима* (доступно на https://www.icmje.org/recommendations/).

ВСП је доступан у режиму отвореног приступа. Сви чланци могу се бесплатно преузети са сајта часописа и користити у складу са лиценцом **Creative Commons Autorstvo-Deliti pod istim uslovima (CC BY-SA) (**https://creativecommons.org/licenses/by-sa/4.0/deed.en**).**

СЛАЊЕ РУКОПИСА

Рукопис рада и сви прилози уз рад достављају се **као један документ** (прилози су инкорпорирани у текст и позиционирани на крају рукописа иза одељка Литература) искључиво електронски преко система за пријављивање *Aseestant*. Ради очувања квалитета фотографија, препоручује се достављање слика и као посебних фајлова, јер Word може смањити њихову резолуцију, како би се избегла компресија слика и евентуални губитак квалитета.

Сви аутори и рецензенти морају бити регистровани корисници система са јединственом е-маил адресом. Регистрацију је могуће извршити на: http://aseestant.ceon.rs/index.php/vsp/user. Техничко упутство за коришћење система електронске пријаве доступно је на: https://aseestant.ceon.rs/index.php/vsp/about/submissions.

Уколико имате проблем са подношењем рукописа путем платформе *Aseestant* можете се обратити за помоћ Редакцији часописа слањем е-мејла на адресу: vsp@vma.mod.gov.rs.

ОПШТА УПУТСТВА

ВСП објављује радове који до сада нису претходно објављени (у целини или делом), који се не разматрају за објављивање нити су прихваћени за објављивање у неком другом часопису.

ВСП не разматра радове који су претходно објављени као препринт верзије.

Часопис прихвата и радове чији су резултати претходно приказани на научним или стручним скуповима и објављени у виду апстракта, под условом да ти резултати нису објављени са DOI бројем (нпр. проширени апстракт у додатку неког часописа).

Уколико је део резултата поднетог рукописа претходно саопштен на научном/стручном скупу или је део докторске дисертације, у Пропратном писму Уредништву потребно је навести званичан назив скупа, место и време одржавања, и да ли су саопштени резултати публиковани и у којој форми (нпр. исти или другачији наслов или сажетак), а у Напомени на крају рукописа то треба посебно назначити.

Радови се објављују на енглеском језику. Поједине категорије радова (нпр. историја медицине/стоматологије/фармације) се по одлуци Уредништва ВСП могу објавити и на српском језику. Све категорије рукописа осим категорија уводник, писмо уреднику, истраживачко писмо, приказ књиге, извештај са научног или стручног скупа се објављују са апстрактима на српском и енглеском језику (у склопу рукописа). О структури и обиму апстракта видети детаљније у одељку Апстракт овог Упутства.

За писање рукописа користити програм *Word*, фонт *Times New Roman*, величину слова 12, проред 1,5. Величину странице подесити на формат А4, са левом маргином од 4 цм а преостале три 2 цм. Текст куцати без дељења речи (хифенације), а после сваког знака интерпункције ставити само један празан карактер. Ако се у тексту користе специјални знаци (симболи), користити фонт *Symbol*.

Подаци о коришћеној литератури у тексту означавају се арапским бројевима у суперскрипту, редоследом којим се појављују у тексту.

Странице нумерисати редом у доњем десном углу, почев од прве стране (изузимајући насловну страну).

При писању текста на енглеском језику придржавати се језичког стандарда *American English*. Обавезно је коришћење међународног система мера (SI). Изузетак чине крвни притисак (mm Hg) i temperatura (°C).

Приликом писања користе се стандардне скраћенице. Избегавати скраћенице у наслову и апстракту осим уколико је неопходно. Пун назив са скраћеницом у загради наводи се у њеном првом помињању, а даље у тексту само скраћенице, како у апстракту тако и у главном тексту. У закључку рада (не апстракта) нема скраћеница.

Не користити комерцијална имена лекова и других препарата, а уколико је то неопходно уз њихове називе обавезно навести и генеричка имена. Уређаји (апарати) се означавају фабричким називима, а податке о произвођачу (назив и место) навести у облим заградама. Уколико се у тексту користе ознаке које су спој слова и бројева, прецизно написати број који се јавља у суперскрипту или субскрипту.

Избегавати фонтове *bold* и курзив (*italic*) јер су резервисани за поднаслове. Изузеци су обавезно писање курзивом оних назива који се тако морају писати (нпр. гени или стране речи - латински).

Групе испитаника морају бити јасно дефинисане и доследно именоване кроз цео рад. За исти појам користити један, јединствен термин кроз цео рад. У одељку Резултати избегавати реченице које почињу са: „Табела X показује “ или „Слика X приказује“. Реченица треба да опише резултат, а ознака табеле или слике да стоји у загради на крају описа. Реченице не би требало почињати скраћеницом, бројем или датумом. Избегавати предугачке реченице које умањују јасноћу текста и дати предност краћим јасним реченицама. Закључак формулисати новим реченицама, без преписивања већ изречених. Превод радова на енглески језик посредством *Google Translate* може изазвати неразумевање текста и стога се не препоручује.

У избору кључних речи користити *Medical Subject Headings – MeSH* (https://www.nlm.nih.gov/mesh/meshhome.html). Кључне речи у прихваћеном рукопису не подлежу ауторској коректури, пошто су оне дескриптори из Тезауруса које одређују стручни индексери.

ОБАВЕЗНА ПРАТЕЋА ДОКУМЕНТА

ИЗЈАВА АУТОРА И АУТОРСТВО

За сваки рукопис који се подноси на разматрање за објављивање у ВСП неопходно је да аутор(и) достави(е) Образац за изјаву о ауторству (Изјаву аутора) да рад претходно није публикован и да није истовремено поднет за објављивање у неком другом часопису, да су рукопис прочитали и одобрили сви аутори који испуњавају критеријуме ауторства, и контакт податке свих аутора у раду (имејл адресу, број мобилног телефона). У овом обрасцу се аутори изјашњавају о сваком могућем сукобу интереса или његовом одсуству. Сви аутори морају Изјаву аутора потписати својеручно.

За додатне информације о различитим врстама сукоба интереса видети препоруке Светског удружења уредника медицинских часописа (*World Association of Medical Editors – WAME;* http://www.wame.org*)*.

ВСП поштује препоруке критеријума за ауторство које даје ICMJE (https://www.icmje.org/recommendations/browse/roles-and-responsibilities/defining-the-role-of-authors-and-contributors.html). Ауторство се заснива на испуњењу сва четири критеријума: значајном доприносу концепцији рада, добијању резултата или анализи/тумачењу резултата; критичкој ревизији рукописа од знатног интелектуалног значаја; одобрењу финалне верзије рукописа која ће бити објављена и преузимању одговорности за све аспекте објављеног садржаја. Сви други учесници који су допринели изради рада, али нису испунили прописане критеријуме требало би да буду наведени у Захвалници уз прецизирање доприноса раду. Потребно је да особе наведене у Захвалници дају писмену сагласност.

ЕТИЧКА САГЛАСНОСТ

Сва истраживања која укључују људе и/или хумани материјал морају бити спроведена у складу са препорукама ICMJE (https://www.icmje.org/recommendations/browse/roles-and-responsibilities/protection-of-research-participants.html) и Хелсиншком декларацијом, ревизија 2024 (https://www.wma.net/policies-post/wma-declaration-of-helsinki/). Скенирану страну дозволе Етичке комисије (ЕК) надлежне институције које је одобрила истраживање, на којој се види датум издавања и предмет истраживања, аутори су у обавези да доставе истовремено са рукописом. Дозвола ЕК се доставља на језику на коме је издата и енглеском језику (може и оверена копија).

У одељку Методе мора бити наведено да је студија одобрена од стране надлежног ЕК, уз навођење назива институције и броја одлуке, као и да је спроведена у складу са етичким принципима за истраживања која укључују људе и/или хумани материјал.

Анонимност пацијената мора бити заштићена у складу са ICMJE препорукама. За сва истраживања која укључују податке о пацијентима који омогућавају директну или индиректну идентификацију, аутори су обавезни да прибаве писани пристанак информисаног пацијента, да у рукопису назначе да је пристанак пацијента прибављен, и да га по потреби доставе Уредништву.

У случају истраживања на животињама, аутори су дужни да доставе одобрење надлежног ЕК који води бригу о поштовању међународних стандарда о употреби лабораторијских животиња у истраживачке сврхе.

Уредништво може одбити радове за које процени да нису изведени у складу са међународним етичким стандардима.

РЕПРОДУКОВАЊЕ ПРЕТХОДНО ОБЈАВЉЕНОГ ЗАШТИЋЕНОГ МАТЕРИЈАЛА И/ИЛИ НЕОБЈАВЉЕНОГ ТУЂЕГ МАТЕРИЈАЛА

Уколико се користе претходно објављене илустрације (фотографије, схеме) уз обавезно цитирање извора преузимања потребно је доставити дозволу (писано одобрење часописа у коме су објављене) за њихову објаву у ВСП. Уколико се користе туђе необјављене илустрације (фотографије, схеме) потребно је доставити дозволу аутора илустрација, за њихову објаву у ВСП.

ПЛАГИЈАРИЗАМ

Од 2012. године сви рукописи достављени на разматрање у ВСП подвргавају се провери на потенцијални (ауто)плагијаризам посредством *SCIndeks Assistant – Cross Check* (*iThenticate*). Рукописи код којих се докаже (ауто)плагијаризам биће одбијени. У зависности од степена и врсте утврђеног (ауто)плагијаризама ауторима се може изрећи забрана објављивања у ВСП-у (различите дужине трајања), уз обавештење надлежних тела у институцијама у којима аутори раде и релевантних професионаних удружења.

КОРИШЋЕЊЕ AI

Генеративна вештачка интелигенција (*artificial intelligence*-AI) или технологије које користе помоћ AI (AI-потпомогнуте) могу се користити само уз поштовање начела транспарентности (употреба AI мора бити јасно наведена у рукопису), одговорности (аутори остају у потпуности одговорни за тачност и оригиналност садржаја), проверљивости (сви учесници у публицистичком процесу морају проверити да AI није унела измишљене податке, цитате или тврдње) и поверљивости (ауторима и рецензентима је забрањено учитавање рукописа поднетих у ВСП у јавне AI сервисе).

Употреба AI алата је допуштена само за ограничене језичке и техничке интервенције у тексту рукописа: исправку граматике и правописа, стилско дотеривање ауторског текста, помоћ при форматирању, техничку асистенцију (попут исправљања кода). Аутори могу користити AI алате искључиво за креирање AI-потпомогнутог, али не и AI -генерисаног садржаја.

Аутори који су користили AI-потпомогнут садржај у обавези су да потпуно и тачно наведу употребу AI алата (тачан назив AI алата, датум приступа, коришћене упите и сврху употребе), гарантују оригиналност научног доприноса, избегавају било какву фабрикацију или манипулацију и поштују правила научне етике. Информације о коришћењу AI се наводе у одељку Методе или Захвалница.

Забрањено је користити AI алате за генерисање већег дела садржаја рукописа, креирање научних идеја, података и резултата, анализу и интерпретацију резултата, формирање закључака, измену слика, табела или графикона (укључујући графичке сажетке), измену података или референци.

Недвосмислено утврђена недопуштена употреба AI за последицу има одбијање рада.

AI ни у ком случају не може бити аутор или коаутор рада, нити може као аутор бити цитиран у одељку Литература.

Ради заштите поверљивости, ниједан део необјављеног истраживања достављеног ВСП не сме бити унет у велики језички модел од стране аутора или рецензента.

Аутори који су користили неки од AI алата су у обавези да приликом подношења рукописа поднесу и Изјаву о коришћењу AI.

ТИПОВИ РУКОПИСА

У ВСП се објављују следеће категорије и типови рукописа и саопштења: уводник, оригинални рад, претходно саопштење, кратко саопштење, приказ случаја и серија случајева, општи (наративни) преглед литературе, мини преглед, систематски преглед литературе, мета-анализа, систематски преглед литературе са мета-анализом, актуелна тема, у фокусу, рад из историје медицине/стоматологије/фармације, писмо уреднику, истраживачко писмо, клиничко истраживање, извештај са конгреса и научног скупа, приказ књиге, *In memoriam* и други прилози.

ОРИГИНАЛНИ ЧЛАНАК

Приказује нова и значајна открића у одређеној области уз детаљан опис коришћених метода истраживања, добијених резултата и изведених закључака. Листа референци треба да укључи најновије и најважније референце из области рада.

ПРЕТХОДНО САОПШТЕЊЕ

Представља приказ истраживања која нису завршена, са налазима који захтевају додатна истраживања и валидацију пре коначних закључака, али су добијене информације од интереса за научну и стручну јавност. Садржи сва поглавља као оригинални научни чланак, али у знатно скраћеном обиму. Аутори се подстичу да касније објаве пуну оригиналну научну студију са комплетним, валидираним подацима и свеобухватном анализом.

КРАТКО САОПШТЕЊЕ

Представља завршено истраживање које је мало по обиму, уско фокусирано са јасним закључцима на основу представљених резултата. Садржи сва поглавља као оригинални научни чланак, али у знатно скраћеном обиму. Сматра се коначном публикацијом тог специфичног, малог истраживања. Не може се поново објавити као чланак пуног обима (иако се подстиче накнадно истраживање које се надовезује на њега).

ПРЕГЛЕДНИ ЧЛАНЦИ

ОПШТИ (НАРАТИВНИ) ПРЕГЛЕД ЛИТЕРАТУРЕ

Преглед, критичка анализа и синтеза постојећих научних сазнања о изабраној теми. Аутори обухватају сву доступну припадајућу литературу за одређени временски период, приказују резултате релевантних истраживања, идентификују недостатке, ограничења или контроверзе и указују на правце будућих истраживања, дајући своје виђење проблема у виду закључног става. Аутори чланка ове категорије могу бити они који су објавили минимално пет радова публикованих у часописима с рецензијом (М20) из области прегледног рада.

МИНИ ПРЕГЛЕДНИ ЧЛАНАК

Сажет преглед постојеће литературе и најновијих достигнућа унутар дефинисаних аспеката одређене истраживачке области и њени нови и/или актуелни правци развоја.

СИСТЕМАТСКИ ПРЕГЛЕД ЛИТЕРАТУРЕ

Синтеза претходно објављених истраживања о одређеној теми коришћењем јасно дефинисаних и унапред одређених методолошких поступака за селекцију и евалуацију. Аутор мора да користи релевантне базе података, постави критеријуме укључивања и искључивања студија и примени транспарентну методологију.

МЕТА-АНАЛИЗА

Користи статистичке методе за комбиновање квантитативних података из више примарних студија како би се идентификовали општи трендови и проценила снага доказа о одређеној теми. Аутор мора да користи релевантне базе података, дефинише критеријуме за укључивање и искључивање и примени транспарентну и репродуцибилну методологију. Неопходно је јасно дефинисање истраживачког питања (PICOS оквир), навођење смерница за одабир и дијаграма тока за селекцију студија (PRISMA).

СИСТЕМАТСКИ ПРЕГЛЕД ЛИТЕРАТУРЕ СА МЕТА-АНАЛИЗОМ

Комбинује квалитативну и квантитативну синтезу, користећи статистичке технике за сумирање квантитативних резултата а квалитативну синтезу за описне/наративне налазе. Аутор мора користити релевантне базе података, јасно дефинисати критеријуме за укључивање и искључивање студија, и применити транспарентну и репродуцибилну методологију. Истраживачко питање мора бити јасно дефинисано према PICOS оквиру, уз навођење коришћених смерница за извештавање (нпр. PRISMA) и укључивање PRISMA дијаграма тока за приказ селекције студија.

АКТУЕЛНА ТЕМА

Разматра савремено, нерешено или контрадикторно питање од теоријског и практичног значаја, уз изношење сопствених резултата истраживања или најновијих важних података из литературе. Конструкција чланка је слободна а пожељне су кратке закључне напомене са јасном поруком.

У ФОКУСУ

Тематска, фокусирана анализа и/или кратак осврт на научни проблем који је у тематској области часописа, а који обрађује питање од значаја за научну заједницу и ширу стручну јавност.

КАЗУИСТИКА

ПРИКАЗ СЛУЧАЈА и СЕРИЈА СЛУЧАЈЕВА (≥4, ≤9)

Приказ случајева са ретком и необичном дијагнозом, дијагностичким процесом, стратегијама лечења, клиничким током, или исходом лечења, који могу бити од користи за клиничку праксу и медицинско образовање. Приликом писања потребно је користити CARE смернице (https://www.care-statement.org/writing-a-case-report). Неопходан је пристанак информисаног пацијента.

УВОДНИК

Уводници су нерецензирани текстови главног и одговорног уредника и/или чланова Уредништва намењени најави новог волумена, тематског броја, садржаја који су од значаја за струку и/или институције чијим члановима је часопис намењен као и уреднички текстови по позиву. Уводници не треба да садрже необјављене или оригиналне податке, а морају укључити изјаву о сукобу интереса.

ПИСМО УРЕДНИКУ

Нерецензирани коментар/критика текста објављеног у ВСП. Пишу се у слободној форми, уз евентуално навођење података из литературе. Не смеју садржати необјављене резултате. Објављују се према одлуци главног и одговорног уредника.

ИСТРАЖИВАЧКО ПИСМО

Кратки приказ оригиналног истраживања, који садржи увод, методе, резултате и дискусију у сажетом облику (без поделе у посебне целине са поднасловима) и максимално до 2 прилога (табеле/слике). Не садржи апстракт и кључне речи али мора да испуни све опште услове за разматрање рукописа (укључујући процес рецензије).

ИСТОРИЈА МЕДИЦИНЕ/СТОМАТОЛОГИЈЕ/ФАРМАЦИЈЕ

Материјал значајан за расветљавање појединих догађаја и/или приказ значајних личности из историје медицине/стоматологије/фармације, а посебно војне медицине/стоматологије/фармације.

КЛИНИЧКО ИСТРАЖИВАЊЕ

Оригинална рандомизована контролисана испитивања и опсервационе студије утицаја једног или више средстава или мера на исход здравља људи, клиничку праксу и здравствену политику. Рукописи морају бити припремљени у складу са међународним смерницама (нпр. CONSORT – https://www.consort-spirit.org/ или STROBE – https://www.strobe-statement.org/) и регистрована у неком од међународно признатих јавних регистара (нпр. ClinicalTrials.gov).

ПРИКАЗ КЊИГЕ

Садржи библиографске податке о публикацији (аутори, изворни наслов, издавач, место и година издања), њен кратак садржај и критичке коментаре садржаја, стила и значаја књиге у датој области. Рукопис не сме бити дужи од 2 странице.

ИЗВЕШТАЈ СА НАУЧНОГ ИЛИ СТРУЧНОГ СКУПА

Приказ активности научног или стручног скупа, уз истицање најважнијих реферата или закључака, односно препорука од значаја за шири круг читалаца ВСП.

ОБИМ РУКОПИСА

Целокупни рукопис рада чине: насловна страна, апстракти на српском и енглеском језику са кључним речима, главни текст рада, захвалност (по потреби), списак литературе, прилози (табеле, слике, графикони, схеме, цртежи).

Обим рукописа за категорије оригинални рад, општи (наративни) преглед литературе, систематски преглед литературе, мета-анализа, систематски преглед литературе са мета-анализом износи до 5 000 речи.

Обим рукописа за категорије мини преглед, претходно саопштење, кратко саопштење, приказ случаја, серија случајева, актуелна тема, клиничко истраживање, историја медицине/стоматологије/фармације износи до 3 000 речи.

Рукописи за остале категорије/рубрике могу имати највише 1 500 речи.

ПРИПРЕМА РАДА

НАСЛОВНА СТРАНА

На првој страници рукописа треба навести следеће:

1. Наслов рада без скраћеница;
2. Пуна имена и презимена аутора (без титула, уз навођење ORCID броја за све ауторе који га имају) са ознакама следећим редом ***, †, ‡, §, ‖, ¶, **, †† …** итд.
3. Пун званичан назив установа у којима аутори раде, место и државу у којој се установе налазе (знаци *, †, ‡, §, ‖, ¶, **, †† … итд.показују редом установе у којима аутори раде);
4. На дну странице навести име и презиме, адресу за контакт, е-маил адресу и број телефона (мобилног/*Viber* или *WhatsApp*) аутора задуженог за кореспонденцију.

АПСТРАКТ

На другој страни рада пишу се апстракт и кључне речи. Апстракт се пише кратким и јасним реченицама. За категорије оригинални рад, претходно саопштење, кратко саопштење, систематски преглед литературе са метаанализом, мета-анализа, клиничко истраживање, апстракт је структурисан и треба да има следеће делове: Увод/Циљ, Методе, Резултати, Закључак. Сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Навести најважније резултате (нумеричке вредности) и ниво статистичке значајности. Закључак мора бити директно повезан са резултатима рада. Обим апстракта не сме да пређе 300 речи.

За категорије приказ случаја и серија случајева апстракт има следећу структуру: Увод (у последњој реченици навести циљ), Приказ болесника, Закључак. Сваки од наведених сегмената писати као посебан пасус који почиње болдованом речи. Обим апстракта не сме да пређе 250 речи.

За остале категорије радова, општи (наративни) преглед литературе, мини преглед, систематски преглед литературе, актуелна тема, у фокусу, историја медицине/стоматологије/фармације апстракт нема посебну структуру и не сме да пређе 200 речи.

Водити рачуна да српска и енглеска верзија апстракта буду међусобно тачни и прецизни преводи. Ниједна реченица не сме постојати у једној верзији а да није преведена у другој.

КЉУЧНЕ РЕЧИ

Испод апстракта навести пет до седам релевантних кључних речи или израза који указују на садржај рада. Препорука је да се не понављају речи из наслова рада. У избору кључних речи користити *Medical Subject Headings – MeSH* (https://www.nlm.nih.gov/mesh/meshhome.html).

СТРУКТУРА ГЛАВНОГ ТЕКСТА РАДА

Неопходно је да оригинални рад, претходно саопштење, кратко саопштење, мета-анализа, систематски преглед литературе са мета-анализом, клиничко истраживање садрже поглавља: Увод (кратак приказ предмета истраживања уз навод циља рада у последњем пасусу), Методе (прецизан опис одабира испитаника и примењених метода, укључујући статистичке методе, број дозволе сагласности надлежног ЕК), Резултати (приказани логичким редоследом без дуплирања приказа истих резултата на више начина), Дискусија (без понављања података који су већ наведени у одељку Резултати; дискутовати само добијене налазе довођењем у везу са другим релевантним студијама, повезати дискусију и закључке са циљевима рада, по потреби нагласити лимитације истраживања), Закључак (који проистиче из резултата датог истраживања), Захвалница (по потреби), Литература.

Рукописи из категорија општи (наративни) преглед литературе, мини преглед, систематски преглед литературе, актуелна тема, у фокусу садрже следеће целине: Увод (са одговарајућим поднасловима), Закључак, Литература.

Рукописи из категорије приказ случаја, серија случајева садрже следеће целине: Увод (циљ рада навести као последњи пасус Увода), Приказ болесника (идентитет болесника мора остати анониман), Дискусија, Литература.

Приказ болесника не сме имати више од пет аутора.

УПИТНИЦИ (Questionnaires)

Сви коришћени упитници који су употребљени као мерни инструменти за било који од испитиваних параметара, морају бити преведени на језик говорног подручја испитаника уз навођење доказа о извршеној валидацији и културолошкој адаптацији поднебљу испитаника.

ПРИЛОЗИ

Прилоге чији број треба да буде усклађен са дужином текста поставити на крај главног текста рукописа иза Литературе, а у самом тексту јасно назначити место које се односи на дати прилог. Крајња позиција прилога биће одређена у току припреме рада за публиковање.

Табеле

Наслов треба написати изнад табеле, а објашњења (легенду) испод ње. Табеле се означавају арапским бројевима према редоследу навођења у тексту. Табеле израдити искључиво у програму *Word*, кроз мени *Table–Insert–Table*, уз дефинисање тачног броја колона и редова који ће је чинити. Куцати фонтом *Times New Roman*, величином слова 12, с једноструким проредом. Табеле морају бити јасне и имати све елементе неопходне за правилно разумевање шта је у њима приказано. Уколико приказане вредности имају „опсег“ или „референтне вредности“, то се мора додати.

У легенди испод табеле треба објаснити све скраћенице наведене у табели и све ознаке (нпр. слова у суперскрипту или болдоване вредности). Такође, неопходно је прецизирати примењене статистичке методе.

Слике (илустрације)

Под сликама подразумевамо све облике графичких прилога (фотографије, цртежи, схеме и графикони). Слике треба уградити у рукопис на крају текста, после литературе и после табела (ако их има). Слике се означавају арапским бројевима према редоследу навођења у тексту. Велика слова А, Б, Ц итд. треба користити за означавање делова вишеделних слика. Слова, бројеви и симболи треба да су јасни и уједначени, а довољне величине да приликом умањивања буду читљиви. Додаци приказани на сликама морају бити сачувани као фотографије (не као измењиви графички елементи), тако да се њихов положај не може мењати, како би се обезбедила тачност података приказаних на слици. Примају се искључиво дигиталне фотографије са минималном резолуцијом од 300 *dpi* и формата JPEG, PNG или PDF. Слике које не задовољаавају наведене услове неће бити прихваћене за објаву. Димензије достављених слика би требало да буду приближне димензијама у којима ће слика бити објављена. Уколико аутори нису у могућности да доставе дигиталне фотографије, онда оригиналне слике треба скенирати у резолуцији 300 *dpi* и у оригиналној величини и као такве их доставити. Сви подаци на схемама и графиконима треба да буду исписани безсерифним фонтом ради лакше читљивости (нпр. *Arial*, *Helvetica*), величина слова не мања од 10 *pt*. Мерне јединице и скале морају бити јасно назначене. Децимални бројеви на графиконима морају бити приказани са тачком, а раздвајање хиљада мора бити означено зарезом (нпр. 1,234.56).

Видео-прилози (илустрације рада) могу трајати 1–3 минута и бити у формату *avi, mp4*(*flv*). Уз видео доставити посебно слику која би била илустрација видео-приказа у е-издању и објављена у штампаном издању, као и линк ка платформи где је видео већ постављен.

У легенди испод илустрација треба објаснити све скраћенице, симболе, бројеве или слова који се користе за објашњење појединих делова слике. У случају графикона прецизирати примењене статистичке методе (по потреби), а код фотомикрографије навести детаље о врсти коришћеног бојења и увећању.

Уколико се приказују фотографије особа (болесника), лик мора бити „замућен“ или је потребно обезбедити писану дозволу лица са фотографије за њено коришћење. На прилозима (снимци рендгена, скенера, ултразвука, итд.) потребно је уклонити све што може да идентификује болесника. Уколико је слика већ негде објављена потребно је цитирати извор уз писано одобрење ако се ради о заштићеном материјалу.

СКРАЋЕНИЦЕ

Скраћенице користити само када је неопходно, и то за веома дугачке називе хемијских једињења, односно називе који су као скраћенице већ препознатљиви (нпр. ДНК). За сваку скраћеницу, осим стандардне јединице мере, навести пун назив при првом навођењу у тексту (укључујући апстракт). У наслову и апстракту избегавати коришћење скраћеница, у наслову их користити само ако су неопходне. За појмове који се у тексту помињу више од три пута препоручује се увођење одговарајућих скраћеница.

ДЕЦИМАЛНИ БРОЈЕВИ

У тексту рада на енглеском језику децималне бројеве писати са тачком (нпр. 22.7), а у тексту на српском језику са зарезом (нпр. 22,7). Кад год је то могуће, број заокружити на једну децималу и писати доследно кроз цео рад (нпр. ако је једна вредност 32.2, све остале морају имати једну децималу, нпр. 32.0).

ЈЕДИНИЦЕ МЕРА

Дужину, висину, тежину и запремину изражавати у метричким јединицама (метар – m, килограм (грам) – kg (g), литар – L) или њиховим деловима. Температуру изражавати у степенима Целзијуса (°C), притисак крви у милиметрима живиног стуба (mm Hg). Резултате клиничких и биохемијских мерења наводити у метричком систему према Међународном систему јединица (SI).

ЗАХВАЛНИЦА

Изнети допринос особе којој треба одати признање, али која не испуњава критеријуме за ауторство. Навести финансијску помоћ (спонзорства, стипендије, опрема и друго), као и назив пројекта у оквиру кога је истраживање спроведено.

СТАТИСТИЧКА АНАЛИЗА

У одељку Методе детаљно описати примењене статистичке методе како би била омогућена провера исправности њихове примене и репродукција анализе. Резултати морају бити нумерички јасно приказани уз одговарајуће показатеље варијабилности и поузданости (нпр. стандардна девијација, стандардна грешка, интервал поверења). Прецизирати тип студије и описати начин на који је изведена. Навести критеријуме укључења и искључења. Навести софтвер и верзију компјутерског програма у коме је извршена статистичка обрада података. У одељку Резултати као и у легендама табела и/или прилога навести статистички метод који је коришћен за анализу приказаних резултата. Вредности *p* се увек пишу са почетном нулом (нпр. $p > 0.05$ а не $p > .05$).

ЛИТЕРАТУРА

Референце нумерисати редним арапским бројевима према редоследу навођења у тексту (укључујући табеле и легенде прилога). Препоручује се да већина цитираних радова буде млађа од десет година. Препоручује се да број цитираних оригиналних радова буде најмање 80% од укупног броја референци, односно број цитираних књига, поглавља у књигама и прегледних чланака мањи од 20%. Сви радови, без обзира на језик извора, цитирају се на енглеском језику, а изворни језик наводи се у загради, иза цитиране референце.

Сви подаци о цитираној литератури морају бити тачни, а цитирани радови лако приступачни читаоцима. Уз сваку референцу навести DOI број. Препоручује се цитирање само радова објављених у часописима које индексирају *Current Contents, Index Medicus* (*Medline*), *Excerpta Medica, Scopus, Web of Science*.

Није дозвољено цитирање апстраката, секундарних публикација, усмених саопштења, необјављених радова, службених и поверљивих докумената, Википедије, препринт објава и *in press* чланака, повучених радова (*retracted article*), радова објављених у предаторским часописима.

Приликом цитирања сајтова, не може се цитирати насловна страна већ се мора цитирати она страна са које је информација преузета. Свака наведена референца мора бити доступна за проверу *online*. Уколико референца не постоји на интернету (нпр. архивски материјал и сл.), аутор мора да достави извор одакле је преузео цитирану литературу односно може сликати или скенирати документ и послати на е-мејл: strliteratura@gmail.com.

Референце се цитирају према Ванкуверском стилу који је успоставио ICMJE (https://connect.ebsco.com/s/article/Citing-Articles-in-Vancouver-ICMJE-Style?language=en_US).

Примери цитирања:

Чланак са 1 до 6 аутора

Nikolić A, Biočanin V, Rančić N, Dušpara M, Đurić D. Serbian translation and validation of the SF-36 for the assessment of quality of life in patients with diagnosed arterial hypertension. EABR Exp Appl Biomed Res 2023; 24(3): 227–34. DOI: 10.2478/sjecr-2020-0073

Чланак са више од 6 аутора

Kapur VK, Auckley DH, Chowdhuri S, Kuhlmann DC, Mehra R, Ramar K, et al. Clinical Practice Guideline for Diagnostic Testing for Adult Obstructive Sleep Apnea: An American Academy of Sleep Medicine Clinical Practice Guideline. J Clin Sleep Med 2017; 13(3): 479–504. DOI: 10.5664/jcsm.6506

Волумен са суплементом

Smith JA, Brown LM. Effects of vitamin D on immune response. J Nutr Sci 2024; 15(Suppl 2): S45–53.

Свеска са суплементом

Zhou Q, Shi R, Kopjar B, Wang H, Chen D, Li H, et al. Adjacent Intervertebral Disc Changes in Patients with Isobar Semirigid Dynamic Stabilization System. Global Spine J 2017; 4(1 Suppl): s-0034-1376699.

Волумен са делом (Pt)

Ozben T, Nacitarhan S, Tuncer N. Plasma and urine sialic acid in non-insulin dependent diabetes mellitus. Ann Clin Biochem 1995; 32(Pt 3): 303–6.

Свеска са делом

Poole GH, Mills SM. One hundred consecutive cases of flap lacerations of the leg in ageing patients. N Z Med J 1994; 107(986 Pt 1): 377–8.

Свеска без волумена

Turan I, Wredmark T, Fellander-Tsai L. Arthroscopic ankle arthrodesis in rheumatoid arthritis. Clin Orthop 1995; (320): 110–4.

Без волумена и свеске

Browell DA, Lennard TW. Immunologic status of the cancer patient and the effects of blood transfusion on antitumor responses. Curr Opin Gen Surg 1993: 325–33.

Пагинација римским бројевима

Fisher GA, Sikic BI. Drug resistance in clinical oncology and hematology. Introduction. Hematol Oncol Clin North Am 1995; 9(2): xi–xii.

Књига

Штампана књига

Ritter JM, Flower RJ, Henderson G, Loke YK, MacEwan D, Robinson E, et al. Rang & Dale's Pharmacology. 10th ed. London: Elsevier; 2023. p. 3630.

Књига у електронском формату

Shreeve DF. Reactive attachment disorder: a case-based approach [Internet]. New York: Springer; 2012 [cited 2012 Nov 2]. 85 p. Available from: http://dx.doi.org/10.1007/978-1-4614-1647-0

Поглавље

У едитованој књизи

Metcalf CS, Smith MD, Wilcox KS. Pharmacotherapy of the Epilepsies. In*:* *Brunton LL, Knollmann BC,* editors. Goodman & Gilman's The pharmacological basis of therapeutics. 14th ed. NY: McGrawHill; 2023. p. 385–411.

У едитованој електронској (online) књизи

Halpen-Felsher BL, Morrell HE. Preventing and reducing tobacco use. In: *Berlan ED, Bravender T,* editors. Adolescent medicine today: a guide to caring for the adolescent patient [Internet]. Singapore: World Scientific Publishing Co.; 2012 [cited 2012 Nov 3]. Chapter 18. Available from: http://www.worldscientific.com/doi/pdf/10.1142/9789814324496_0018

Веб страница

Интернет страница

Diabetes Australia. Diabetes globally [Internet]. Canberra ACT: Diabetes Australia; 2012 [updated 2012 June 15; cited 2012 Nov 2]. 85 p. Available from: http://www.diabetesaustralia.com.au/en/Understanding-Diabetes/Diabetes-Globally/

Део интернет странице

Australian Medical Association [Internet]. Barton ACT: AMA; c1995-2012. Junior doctors and medical students call for urgent solution to medical training crisis; 2012 Oct 22 [cited 2012 Nov 2]; [about 3 screens]. Available from: https://ama.com.au/media/junior-doctors-and-medical-students-call-urgent-solution-medical-training-crisis

Зборник радова са конгреса

Kimura J, Shibasaki H, editors. Recent advances in clinical neurophysiology. Proceedings of the 10th International Congress of EMG and Clinical Neurophysiology; 1995 Oct 15–19; Kyoto, Japan. Amsterdam: Elsevier; 1996.

Рад из зборника

Bengtsson S, Solheim BG. Enforcement of data protection, privacy and security in medical informatics. In: *Lun KC, Degoulet P, Piemme TE, Rienhoff O,* editors. MEDINFO 92. Proceedings of the 7th World Congress on Medical Informatics; 1992 Sep 6–10; Geneva, Switzerland. Amsterdam: North-Holland; 1992. p. 1561–5.

Дисертација

Knežević D. The importance of decontamination as an element of complex therapy of poisoning with organophosphorous compounds [Ph.D. Thesis]. Belgrade: School of Veterinary Medicine; 1988. (Serbian)

Остали објављени чланци

Новински чланак

Vujadinović J. The inconsistency between federal and republican regulation about pharmacies. In between double standards. Borba 2002 February 28; p. 5. (Serbian)

Свето писмо

Serbian Bible. Belgrade: British and Foreign Biblical Society; 1981. Book of Isaiah 2: 19–22. (Serbian)

Речници и сличне референце

Kostić AĐ. Multilingual Medical Dictionary. 4th Ed. Belgrade: Nolit; 1976. Erythrophobia; p. 173–4.

Остале примере навођења публикација можете видети на https://www.nlm.nih.gov/bsd/uniform_requirements.html

НАКНАДЕ ЗА ОБЈАВЉИВАЊЕ ЧЛАНКА

Накнада за објављивање рада се плаћа након прихватања рукописа за објављивање. Одлука о прихватању мора бити потврђена на седници Уредништва ВСП. Радови за које нису плаћене накнаде неће бити објављени.

Уплата није гаранција да ће рад бити објављен у ВСП.

Сви аутори дужни су да плате *Article Processing Charge* за покриће трошкова језичке, стручне и техничке обраде рукописа, као и његовог објављивања. Домаћи аутори плаћају износ од 5 000 динара, а инострани 150 еура.

Институционална претплата (10 000 динара) важи искључиво за институције (установе) које желе да добијају физичке копије месечних бројева часописа.

У години у којој су уплатили претплату аутори могу поднети неограничен број радова у ВСП.

Копије уплатнице неопходно је доставити на е-мејл адресу: strliteratura@gmail.com ради евиденције уплате.

Од претплате су ослобођени рецензенти ВСП (потребно је доставити ID број рада или наслов рада који су рецензирали), студенти на докторским студијама (уз потврду о уписаној текућој години), уредници. Уредништво задржава право да може умањити износ претплате или ослободити ауторе плаћања претплате на образложену молбу аутора.

За све додатне информације, можете се обратити на доле наведене адресе и бројеве телефона.

Редакција часописа „Војносанитетски преглед“
Ул. Црнотравска 17
11 000 Београд
Србија
Телефон: (+381 11) 3608-997
Е-пошта: vsp@vma.mod.gov.rs
Е-пошта за информације у вези са претплатом: strliteratura@gmail.com
Интернет страница: www.vsp.mod.gov.rs
ISSN 0042-8450
ISSN Online 2406-0720 OPEN ACCESS